THE BRITISH VETERINARY JOURNAL

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EDITORIAL

"There are more things in heaven and earth, Horatio,
than are dreamt of in your philosophy."
—*Hamlet:* I, V, 166.

THE Bard of Avon made words ring like mellow bells whose reverberations have encircled the world and brought glory to the English tongue. His golden voice and connotation reveal a depth of understanding, vision and foresight worthy of a great philosopher. He lived in a time when the dark ages still cast their shadows and when life was primitive by modern standards. If a man of Shakespeare's time, or of a much later date, even of the nineteenth century, could be translated to the year 1956 it would be strange if he were not bemused and perplexed by what he could see and hear, for many things which are accepted now as commonplace would be far beyond his philosophy. Men speak to one another from the farthest corners of the world as easily as they could address one another in the street, and their eyes can witness great occasions occurring many miles away and depicted with remarkable clarity on a screen in their home.

The advance of knowledge has enabled conjecture to be replaced by fact: and there would seem to be little place in the modern world for the prophetic musings of those who just sit and think.

It is a remarkable fact that many of the factual wonders of our day were projected in the recorded observations of savants many years ago. Often their

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words were received with ridicule and suspicion even on those occasions when their visions were implemented in fact, for the world was not ready for their reception. The steam engine, the internal combustion engine, ships made of iron, the telephone and wireless, both sight and sound, all met with their quota of ridicule and suspicion and their full development suffered delays. On the whole men are opposed to change and especially so when new ideas seem to contravene upon previous accepted practice or belief. This attitude is good in many ways for one should not abandon lightly that which has served well. On the other hand, a too-rigid acceptance of such principles may do great harm for it engenders orthodoxy, and loyal compliance with accepted views may lead to the denial of progress. The history of medicine offers many instances of this brake on scientific advancement. The inflexible adherence to the precepts of Galen thwarted progress for many centuries and in more recent times new ideas and discoveries in anæsthetics, surgery and therapeutics have been vigorously opposed. Fleming had to stay in the wilderness for nearly twenty years before his life-saving antibiotic broke through the barriers of opposition.

All new things are not necessarily sound and good. All research work originates from some presupposition or idea. The object of the subsequent work is to prove or disprove the original hypothesis. If the hypothesis fails all is not lost for the worker is often led to another trail which may prove fruitful. The great danger is that an enthusiastic worker may be so imbued with the original idea that his investigations may be biased towards its proof. If this be so it is not a cogent reason why one should condemn his work and suppress its publication.

The world is too short of original thinkers and those who are brave enough to leave the beaten track for their work to be cast aside with disdain. In this issue readers will find an article by a worker who holds unorthodox views and opinions relating to the treatment of disease of germ origin. We publish because we subscribe to the opinion that all men have a right to express their views be they unique or otherwise. We recognise that the article may excite comment. That comment may be favourable or unfavourable and our columns are open to free and frank discussion. In that way may truth prevail.

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GENERAL ARTICLES

RAGWORT POISONING FROM SILAGE

By L. G. DONALD and P. L. SHANKS

Veterinary Investigation Laboratory, The North of Scotland College of Agriculture

MANY cases of Ragwort (*Senecio jacobœa*) poisoning in cattle occur annually; the Ragwort may be consumed directly from the grazing or indirectly via a crop. Cattle do not normally eat Ragwort in the growing stage but they will consume the plant if the pasture is bare and no other foodstuffs are provided. It is well known that cattle will eat hay containing Ragwort and that poisoning may follow its ingestion, e.g., Stockman, 1917; Leyshon, 1926; Craig *et al.*, 1930; Cartwright, 1936; Reynolds, 1936; Bisset, 1936; Lofthouse, 1949. Recently Cockburn *et al.* (1955) reported an outbreak of Ragwort poisoning attributable to the feeding of cubes containing lucerne contaminated with Ragwort. It appears, however, that many stockowners and others do not appreciate the danger of feeding silage containing Ragwort although there have been reports in the literature (Ferguson, 1940; Kerr, 1953). Forsyth (1954) has stated that "Ragwort alone probably causes more annual loss to the livestock industry than all other poisonous plants put together" and remarks further upon "the enormous quantities which grow in pastures throughout the country." Cockburn *et al.* (1955) also referred to "the growing menace of Ragwort infestation" and Gould (1954) commented upon "the extraordinary increase in the prevalence of Ragwort" in that year. Thomas (1955), discussing botanical changes following the great reduction of rabbits from myxomatosis, reported that Ragwort had grown in great profusion in some places during the summer months. The above facts prompted the writing of this article describing an outbreak of Ragwort poisoning in a large group of young cattle which were fed on silage containing a considerable quantity of Ragwort. The farmer has estimated his over-all loss in thousands of pounds.

History

On November 1, 1954, 249 weaned cross calves (6 to 8 months old) were housed in ten covered courts; a further two courts were filled with 22 yearling cross bullocks. All these cattle had spent the summer and early autumn on a nearby farm (belonging to the same owner) where Ragwort infestation was not a problem.

The feed in the courts consisted of draff (wet brewer's grains), bruised oats, cake, turnips, silage and straw *ad lib.* Silage was introduced on November 10 at the rate of 16 lb. per head per day; all the cattle ate the silage although at first they were reluctant to do so.

On January 1, 1955, all stock was known to be in good thriving condition. A fortnight later, however, one stirk was ill and was treated for a digestive upset; it survived until February 2. In the meantime, other stirks in different courts had become ill and died within 48 hours. Between January 18 and 23, five animals died and losses continued sporadically thereafter for several months involving both yearling and two-year-old stock.

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At first, Ragwort poisoning was not suspected and the cause of death was not ascertained. We were called in at the end of January to make an investigation and on February 1 one of us (P.L.S.) examined the stock and made an autopsy. The post-mortem findings and clinical signs suggested the possibility of Ragwort poisoning and inquiry revealed that the silage contained a considerable quantity of Ragwort. The feeding of silage was stopped immediately. Until an analysis had shown that lead was absent, the draff and cake were withheld; the turnip ration was increased and hay was introduced.

Progress of Outbreak

The farmer was informed that he would probably incur losses for several weeks and possibly even for months in spite of discontinuing the feeding of the contaminated silage. He decided, however, not to salvage the remaining stock immediately but to await developments and slaughter as many animals as possible whenever symptoms appeared.

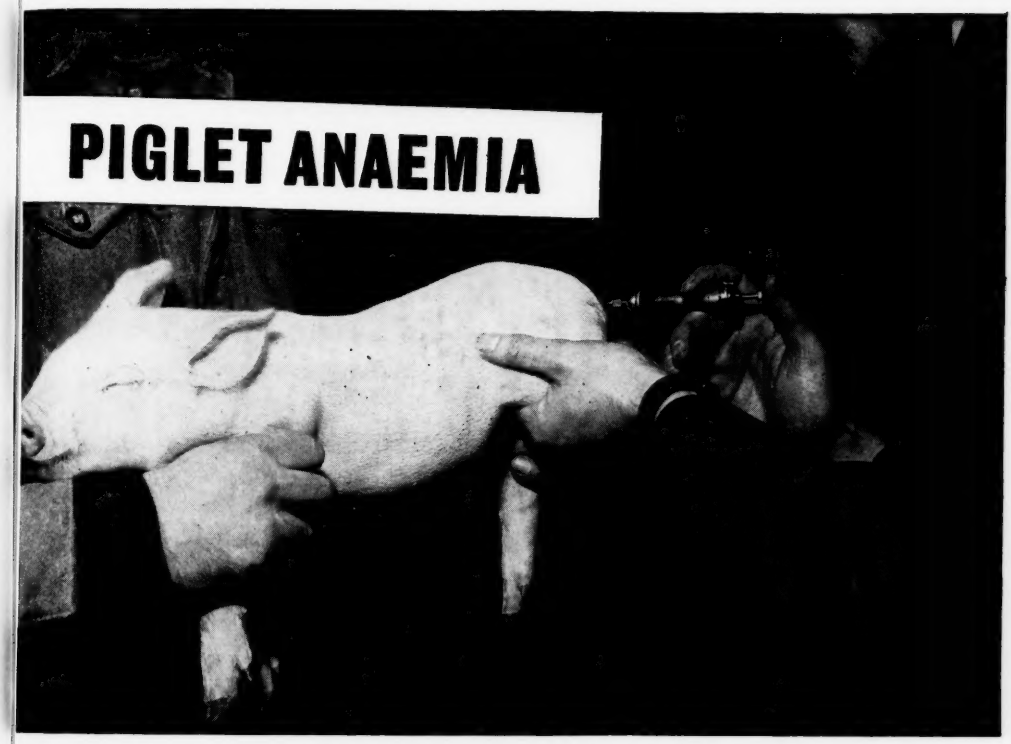
By April 15, when the young stirks (now yearlings) were put out to grass, 36 of the original 249 had died; but worse was to follow, for within 48 hours of leaving the courts a further 12 yearlings died, bringing the total to 48. Cases occurred sporadically thereafter until the end of June, by which time 57 yearlings had been lost and 28 had been salvaged, making a total casualty figure of 85 (or 34 per cent of the original number). The livers of all 28 salvaged beasts were condemned because they were affected with cirrhosis.

No further deaths occurred in the yearlings after June, but in October and November it was found necessary to salvage three that had been unthrifty. Liver abscesses were found and the livers were condemned on this account. Cirrhosis was not recorded but, as abscesses had been found, an examination for cirrhosis was not made by the detention officer.

In September, 70 yearlings were sold as stores. In November and early December the remainder were sent for slaughter; they weighed between $8\frac{1}{2}$ and $9\frac{1}{2}$ cwts. Twelve of the slaughtered animals were examined by us in the abattoir. Cirrhosis was evident to the naked eye in five livers, but no gross changes were found in the other seven. Histologically, cirrhotic tissue was present in all 12 livers to a varying degree. No other abnormalities were observed.

Only one of the 22 two-year-old bullocks died in the courts (April 18), but three died outside between May 20 and June 21. Four were salvaged in early summer (after developing symptoms of Ragwort poisoning) and the remaining 14 were sold fat (10 to 12 cwts.) in September. None of these animals was examined post-mortem by us, but it is known that the livers of the four salvaged bullocks were condemned on account of cirrhosis.

The bullocks and heifers were segregated in different courts. It was observed that almost invariably the smallest animals in each court were affected first and that more bullocks than heifers were affected, particularly after the animals had been put out to grass. During the outbreak 58 bullocks (40 per cent) and 35 heifers (27.5 per cent) died or were salvaged out of the original totals of 144 and 127 respectively. These figures are significant ($P = 0.05$). It is of interest



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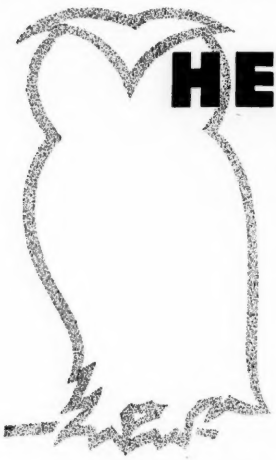

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that Smit (1952) found that male rats were more susceptible to *Senecio* alkaloids than females.

Symptoms

The symptoms varied according to the duration of the illness. Some animals died within 24 hours, whereas other cases lingered for days or even weeks. None recovered once symptoms were shown.

The first sign often observed was an unsteadiness of the hind limbs and a tendency to trail, rather than lift, the hind feet when walking; the appetite was poor and there was a loss of condition. The eyes became glazed and, in the later stages, the animal was apparently blind. Both scouring and constipation occurred.

Continuous straining was a common feature and many showed prolapse of the rectum. Some developed a mania and charged any person approaching. An unusual symptom observed on two or three occasions in this outbreak, and one which came to be regarded as imminent of death, was walking in small circles. An affected animal always turned in the same direction. Pallor of the visible mucous membranes was always seen in cases examined clinically. In the later stages abdominal pain was evident and the animals became comatose prior to death.

Treatment was not attempted after the diagnosis had been established.

Post-mortem Findings

Twenty-two yearlings were examined post-mortem by us. In the acute cases, early in the outbreak, a striking feature was the presence of petechial hæmorrhages scattered throughout the subcutaneous tissue and organs somewhat resembling the findings in acute bracken poisoning. These lesions were not observed later in the outbreak. Ragwort (Senecio jacobœa) was identified in the rumen of the early cases.

All post-mortem examinations, including those of the early acute cases, revealed small yellow livers showing varying degrees of cirrhosis. Œdema of the wall of the abomasum and ascites were frequently observed. In two cases œdema of the mesentery was noted particularly in the part adjacent to the intestine. One of the stirks showed petechiæ scattered diffusely in the mucous membrane of the abomasum.

Laboratory examinations. Bacteriological examination of the tissues did not reveal the presence of any known pathogens and material submitted for chemical analysis yielded no significant information. Histo-pathological examination of the liver showed a pericellular cirrhosis which varied in degree in different cases.

Silage

More than 300 tons of grass silage was made in June, 1954. One field of second-year grass was infested with Ragwort and the grass off this field was ensiled on top of that from the other fields which were free from Ragwort. Some of the Ragwort had reached the flowering stage at the time of cutting. A second cut was taken from the same fields in July and ensiled separately from the first cut.

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For feeding, the silage was cut vertically from the silage pit so that equal amounts of Ragwort-free and Ragwort-contaminated silage were fed over the 12-week period from early November to early February. It was estimated that the amount of Ragwort in the contaminated silage was about 25 lb. per ton and the amount of that silage consumed daily was about 8 lb. per head. Therefore, the total amount of Ragwort taken by each stirk between November and February averaged 7.5 lb. These figures are, of course, estimations based on rough calculations.

In view of the losses involved, the farmer decided to discard the whole of his second cut of silage and also the top layer of his first pit, i.e., the layer which contained Ragwort plants. He decided to risk feeding the lower layer of silage to a few cows and bullocks, hoping that no Ragwort alkaloids had percolated from the top layer. No ill effects were ever noticed in these animals.

It is of interest to record that silage was made from the same fields in 1953; Ragwort was present but the infestation was not so heavy as in 1954. Ragwort poisoning was not diagnosed during the winter of 1953-54 but three animals died and, according to the symptoms, it now appears possible that Ragwort poisoning had been the cause of the deaths.

Sheep grazed on Ragwort-infested pasture.

A flying flock of hoggs was put on the Ragwort-infested pasture in April, 1955, in order to control the weed. The hoggs cleaned the grazing in a satisfactory manner and were sold in July in very good condition. It has been shown, however, that Ragwort can be toxic to sheep (Eadie and Robinson, 1953), and the timely disposal of the flock may have prevented a further catastrophe. Steyn (1934) reported that the ingestion of other *Senecio* species may also prove fatal to sheep.

Discussion

It is known that Ragwort is toxic to cattle when ingested over a period of time. On the other hand, the insidious nature of an outbreak of Ragwort poisoning may tend to diminish, in the mind of the stockowner, the serious consequences that may result from its inclusion in the diet. Further, an impression may be held that because cattle do not normally eat the growing plant they will also reject it if included in hay, silage or other crop. Yarded cattle, however, generally have no choice in the foodstuffs that they consume and Ragwort, if present in the feed, will be eaten. As Ragwort is very prevalent in this country it is certain that many crops of hay and silage will contain the plant, particularly if the farmer is apathetic or ignorant of the consequences. A small amount appears to do no harm. In the outbreak described above, the farmer was aware that Ragwort may poison cattle but did not realise the significance of making silage from a field heavily infested with the weed. The present observations and those recorded previously (Ferguson, 1940; Kerr, 1953) indicate that the alkaloids of Ragwort are not destroyed in the ensiling process. It has not been demonstrated what is the minimum amount of Ragwort in silage that is necessary to cause death, but the stirks in this instance ate an estimated average of 7.5 lb. over the 10-week period. Some stirks no doubt consumed more than others,

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but it is not known if the greedy feeders were the first to succumb. (The evidence actually points against this since it was mainly the small stirks that died at the beginning of the outbreak. In the absence of experimental evidence it seems that the criterion may be the proportion of the amount of alkaloid ingested to body weight; the bigger the animal the more alkaloid that is required to produce a toxic effect. Alternatively, the older animals may be more tolerant.) One interesting observation made by the owner was that in late summer many of the surviving stirks were heavier than usual. Is it possible that the Ragwort had stimulated the liver? Other factors may, of course, have influenced the rate of growth and a controlled experiment would be necessary to substantiate the observation.

The long latent period is a feature of Ragwort poisoning and fresh cases were still arising in this outbreak 5½ months after the contaminated food had been removed. Furthermore, five of the 12 yearlings slaughtered nine months afterwards showed gross liver cirrhosis. The turning-out of the cattle to grass appeared to precipitate further losses, 12 stirks dying within 48 hours. During an outbreak emphasis might, therefore, be placed on the need for avoiding excitement and stir at this time. It is probable, however, that even had the stirks stayed in the courts death would have supervened within a few days due to the advanced cirrhosis of the liver. There were no losses in the flock of hoggs bought for the purpose of cleaning the Ragwort pastures; the hoggs were bought in mid-April and sold off at the end of July.

Summary

An outbreak of Ragwort poisoning is described involving 249 yearling stirks and 22 two-year-old cattle fed on silage containing Ragwort. Silage feeding began in November, 1954, and the first case occurred ten weeks later in January. The silage was discontinued immediately after the diagnosis was established, but nevertheless 61 cattle died and 32 more were salvaged during the following 5½ months. Four months later, five out of 12 slaughtered animals showed gross liver cirrhosis. The farmer estimated his over-all loss in thousands of pounds. Emphasis is placed on the danger of making silage (or hay) when Ragwort is present in the pasture.

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XUM

THE PREVENTION AND CURE OF VIRUS DISEASES WITH MICROBIAL ANTIGENS*

By W. M. CROFTON, M.D.

MR. PRESIDENT, Ladies and Gentlemen: I must tell you at once how very much I appreciate the invitation to address your very distinguished Society.

I am glad to have this opportunity to make a public acknowledgement to members of your profession, Mr. Orr and Miss McLelland in England, Dr. Argenti in Uruguay, numerous docteurs veterinaires in France, and Mr. David O'Brien, now in the veterinary department of Tasmania, who have given me such brilliant collaboration in my effort to demonstrate the specific method of prevention and especially the cure of dire infections, such as hardpad distemper.

I will speak of the cure of acute and chronic infections with dead antigens of the causative microbes, the most powerful, accurate and specific method of cure ever devised. These antigens are superior to the anti-toxic serum method for diphtheria, for when a bacillary diphtheria antigen is used no carriers are left as they are with the anti-toxic serum and Toxoid method.

Most people seem to agree that it is quite reasonable to use microbial antigens, living or dead, for the prevention of disease but not as a cure. The late Professor Okell said: "By adding more of the poison, even if modified by killing, to a body having all it could do to resist the disease, was one of the most absurd conceptions of the human mind, and there was no evidence in either man or animals that any disease had been even relieved, much less cured, by such a method." In my book, "The Cure of Acute and Chronic Infections by Active Immunisation," I attempted to show how wrong Okell and his colleagues were and that they were not taking into account the physiology of immunity, and the natural history of microbes. I pointed out that if Okell was right he would be particularly right in the treatment of acute infections. I am now going to show you some slides illustrating specific and dramatic cures obtained by this method in acute infections. I used some of these slides in my lectures at University College, Dublin, and also demonstrated them in a communication to the Pathological Society.

Examination of these lantern slides will show that these antigens, simple killed suspensions of the causative streptococci, cured very dire cases of puerperal fever.

The cases were treated at the Rotunda Hospital in 1911 by my late friend and rival Robert Rowlette during the mastership of the greatest of all the Masters, my friend and ally Hastings Tweedy. One patient had a temperature of 106.8 in her armpit. She was alive 30 years later.

The distinguished work of Rowlette, who reduced the morbidity and mortality dramatically at the Rotunda, seems to be forgotten.

* Address and subsequent discussion at a meeting of the Central Veterinary Society, December 1, 1955.

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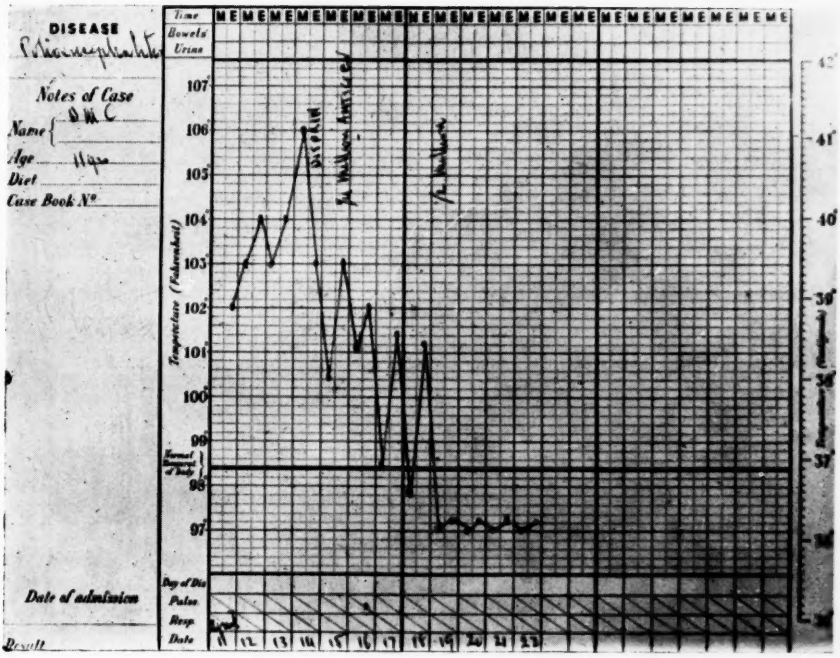

PLATE I

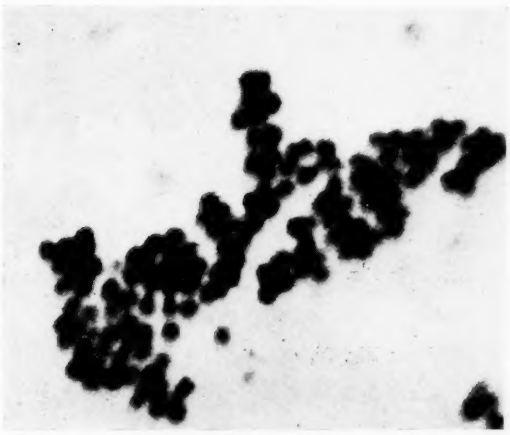

PLATE I A

(*Article by Crofton, page* 312)

LEGENDS TO ILLUSTRATIONS

Figs. 2, 3 and 4.—Copeman's distemper bacilli grown from three samples of virus issued for prophylaxis with contaminating bacilli. Such material is always impure.

Fig. 5.—Distemper bacilli sprouting from spheroids which appear to develop first from the virus.

Fig. 6.—Culture from intact vesicle material from Switzerland. White colonies staphylococci, grey streaks confluent foot-and-mouth bacilli.

Fig. 7.—Piece of cow's tongue from Belgium. Mixed culture of a few Grampositive bacilli and large numbers of foot-and-mouth bacilli.

Fig. 8.—This culture plated out showing: (*a*) foot-and-mouth bacilli; (*b*) large Grampositive bacilli.

Fig. 9.—Foot-and-mouth bacilli of Fig. 7 with some large Grampositive baccili.

Fig. 10.—Pure culture of foot-and-mouth bacillus.

Fig. 11.—Clear filtrate from cow's blood infected with foot-and mouth bacillus.

Fig. 12.—Culture of pure foot-and-mouth bacillus, from Fig. 11.

Fig. 13.—(*a*) Pure culture of foot-and-mouth bacillus from Fig. 12; (*b*) pure foot-and-mouth bacilli which developed slowly in filtrate from Fig. 11.

Fig. 14.—Vaccinia microbe. Coccobaciliary forms. First subculture from rabbit's nasal mucous membrance.

Fig. 15.—Vaccinia microbe. Several subcultures away from those in Fig. 17.

Fig. 16.—Skin of rabbit 3, showing vesicles.

Fig. 17.—Skin of rabbit 4, showing typical confluent vaccinia, with outlying vesicles.

Fig. 19.—Tumour at site of subcutaneous inoculation.

Fig. 20.—Secondary tumours in lung.

Fig. 21.—(*a*) Lung tumour. 1/6 obj.: (*b*) J.R.S. 1/12 obj.; (*c*) tumour at site of inoculation. 1/12 obj.; (*d*) lung tumour. 1/12 obj.

Fig. 22.—Sarcoma of thymus.

Fig. 23.—Bacilli grown from Fig. 22.

Fig. 24.—Myelæmic spleen.

Fig. 25.—Bacilli grown from Fig. 24.

Fig. 26.—Lymphosarcoma.

Fig. 27.—Bacilli grown from Fig. 26.

Fig. 28.—Culture from infiltrating carcinoma of ovary. Piece of tumour removed by laparotomy.

Fig. 29.—Culture from early scirrhus of breast.

Fig. 30.—White staphylococcus from Fig. 28. Doyen's micrococcus noeformans. Identical microbes grew in culture Fig. 29.

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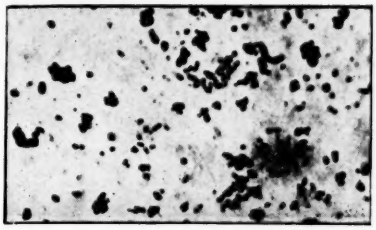

Fig. 2

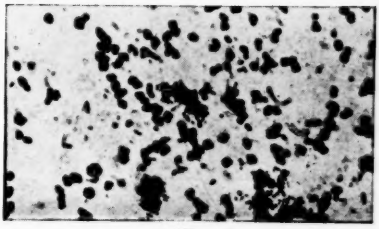

Fig. 3

Fig. 4

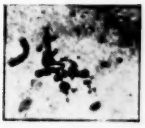

Fig. 5

Fig. 6

Fig. 7

Fig. 8

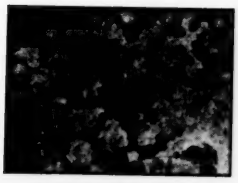

Fig. 9

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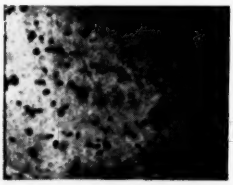

Fig. 10

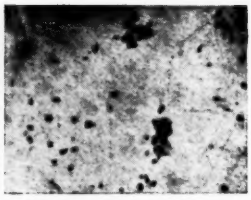

Fig. 13(*a*)

Fig. 12

Fig. 11

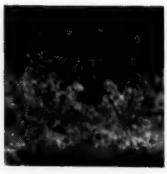

Fig. 13(*b*)

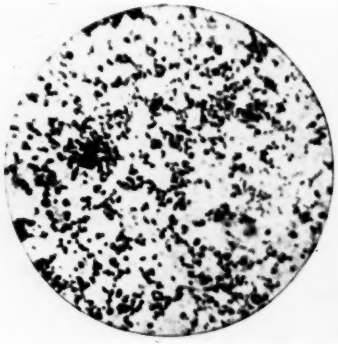

Fig. 14

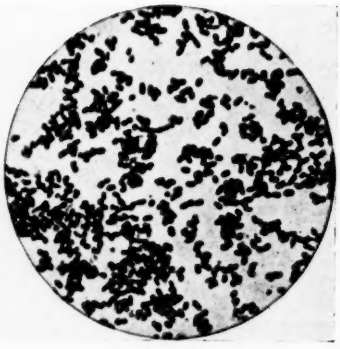

Fig. 15

Fig. 16

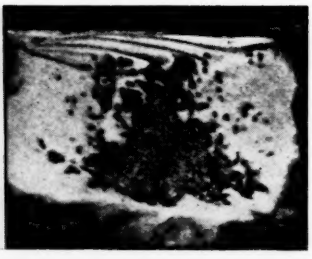

Fig. 17

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Fig. 19

Fig. 20

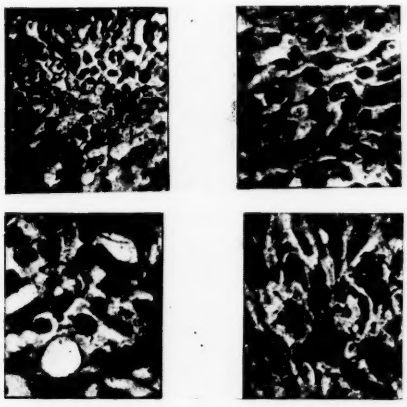

Fig. 21 (*a*) (*b*) (*c*) (*d*)

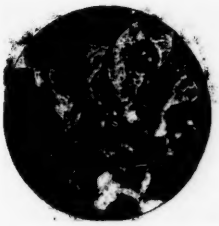

Fig. 24

Fig. 25

Fig. 26

Fig. 27

Fig. 28

Fig. 29

Fig. 22

Fig. 23

Fig 30

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This slide (Fig. 1) tells of a very severe case of septicæmia which was cured by immunisation at Dr. Steevens's Hospital in 1912. Sgt Gallagher of the Royal Irish Constabulary was going up Croagh Patrick on the Annual Pilgrimage when he slipped and grazed his left knee. Within three days he was in hospital

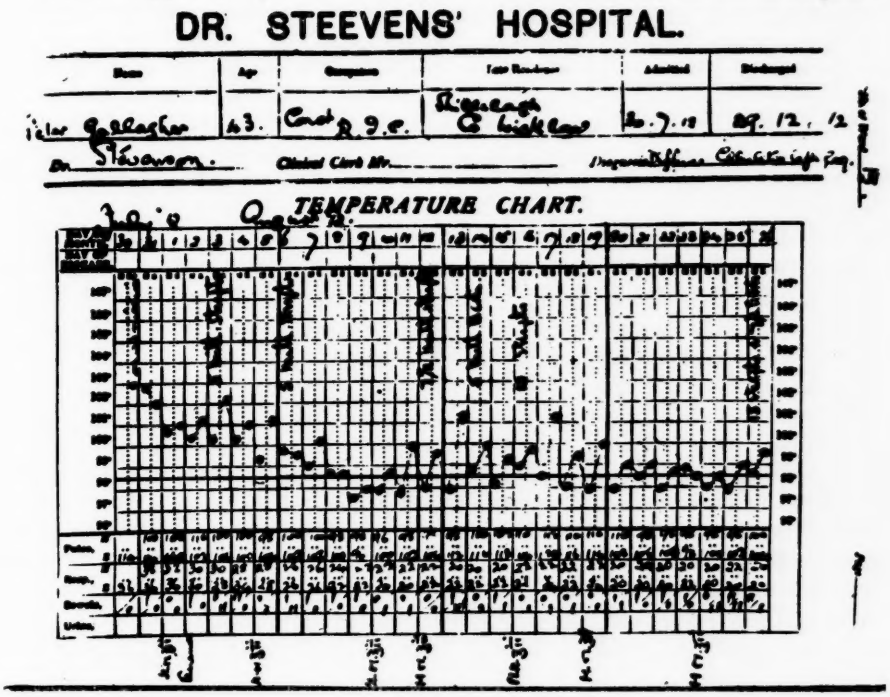

Fig. 1

and delirious. His knee was opened and several other openings were made in his leg. Mr. Stevenson, the Surgeon, asked for my help. The Sergeant recovered and left hospital with a perfect leg.

In the pre-war (1914-18) years and during the shocking civil war in Ireland, my surgical colleagues and I lost neither life nor limb from sepsis. The Surgeons did the minimum essential surgery that I asked them to do and I treated the patients by immunisation. Four great colleagues, Swan, Houghton, Stevenson and George Pepper gave me every encouragement. The last is the only one alive now.

Now I am going to show you some slides of acute, so-called, virus diseases cured by microbial antigens. In order that you may understand my methods, I will give you a little demonstration of the bacteriological technique involved and emphasise that in my experience there is no such thing as a virus of its own kind. All of them are the form they assume whilst they are in the tissues. The microbes are the form they assume when growing in air. To obtain potent antigens it is necessary to grow the microbes on a living tissue. The nearest we

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can get to this is unheated blood-agar. Here is a tube of human blood-agar. I developed this medium in 1910 and demonstrated it in Ireland at the Royal Academy of Medicine. Bacteriologists knew that unheated blood would kill the microbes. Such a medium is necessary, for if there is no living tissue to live on, the microbes lose their ferments, which we call toxins, and the antigens lose their potency materially. These slides demonstrate that in these so-called virus diseases a dead microbial antigen has produced a cure. The first case was one of influenza pneumonia in a little 3-year-old girl. I gave her a dose of mixed influenza antigen, but her temperature went up again and the autogenous antigen, which I had rushed through, arrived just in time. The death rate from influenza pneumonia was very high at that time, but even in the dreadful epidemic of septic-pneumonia during the winter of 1918-19 I did not lose a single patient. One case was rushed into Dr. Steevens's Hospital by a former pupil. The temperature was over 112 degrees. The patient responded perfectly to immunisation with a pure influenza antigen. The statement that the Pfeiffer's bacillus was absent in the summer of 1918 is contrary to the fact. If those who denied its presence because they could not grow it had used blood-agar medium they would not have made this mistake. In June, 1918, C. J. Martin grew it in every one of 50 cases at a base hospital in France and published the facts in the *B.M.J.* in September of that year. I recorded my observations in the *Lancet* in March, 1919, and in numerous previous communications. These facts are quoted in my book "The True Nature of Viruses." I would like you to look at some slides illustrating the cure of two very severe cases of (*a*) polioencephalitis (Plate 1 & 1*a*) and (*b*) bulbar poliomyelitis, which were both cured by using the dead microbic phase of the causative organism. For nearly 40 years I have grown this microbe (Fig. 3) from the patients' urine and have made a dead antigen from it.

It would appear that a dead microbial antigen exercised a most potent curative power in these so-called virus diseases. If the microbe had not been the causal agent the antigen would have had no effect upon the course of the disease.

The work on influenza conducted by the Medical Research Committee (the predecessor of the Medical Research Council) was initiated in the hope that it would lead to the discovery of a potent antigen for the disease. The members of the committee assumed that distemper was a virus disease in spite of the fact that Professor Ferry of the U.S.A. had published a paper in the Journal of Pathology and Bacteriology in 1915 describing his most careful and controlled experiments. He had grown the Copeman bacillus in media which allowed it to go into a filterable phase. His decisive paper was ignored in 1919 and has been ignored ever since. The statistics of the results where live virus vaccine is used should be published. I expect that most of you have experienced some disastrous results. The Frenchman, Carré, announced the discovery of the virus in 1910 and said that he had failed to grow the Copeman bacillus. I say that any competent bacteriologist should be able to grow the Copeman bacillus from any case of distemper and also that he should be able to grow the bacillus from the live virus. Instead of putting a dose of the egg-adapted virus into a dog, put it on a blood-agar slope and into an incubator. There will be a profuse growth

of the Gram-Negative Copeman Bacillus the next day (Figs. 2, 3, 4 & 5). It will not be quite pure, it never is. To say this bacillus is a contamination is not fair to the manufacturers. If it is alive and one injects it into a puppy then you are quite likely to give the puppy distemper or encephalitis or both. If you suspend it in 2 per cent carbolic in tap water, and after an hour inject a proper dose into a puppy, that puppy cannot get distemper. The puppy will not be infectious for a fortnight or more and it is protected almost at once. My pupils have found, as the followers of Copeman found, that the dead bacillary antigen reduced the morbidity and mortality from distemper almost to nothing.

Official investigators have never suggested the use of dead bacillary antigen for treatment. You know from Miss McLelland's report in the *Veterinary Record* of the work she and Mr. Orr did in a series of cases of hardpad distemper. She reports the turning of an 86 per cent death rate into an 86 per cent recovery (*Vet. Rec.*, October 18, 1952). These figures are very conservative for they include dogs that were moribund when the first dose was given, for I asked them to treat every affected dog that was presented, even the apparently hopeless cases. We cannot be accused of choosing cases. I have given you reasonable evidence of the cure of some virus diseases by the use of dead microbial antigens. Foot-and-mouth disease is attributed to a virus. Foot-and-mouth disease is a very easy disease to treat. The ordinary death rate is only 2 per cent or less, so one can give the average sick beast a good dose of the dead bacillary antigen without any fear of killing it. Recovery is complete within a few days and the affected animal is not a carrier.

I will show you some slides which illustrate how easy it is to grow the bacillus of foot-and-mouth disease. This slide shows the growth from samples of V.O.I. strain sent to me from Pirbright in 1939. I grew this bacillus again from the same sample 14 years later in order to show it to the Gowers Committee. The sample had been lying in my laboratory winter and summer for 14 years. You will note what a tremendous survivor this organism is. It is quite easy to turn it back into its virus phase by incubating it for 24 hours in fresh defibrinated blood. Then I filter it through an asbestos disc to obtain the clear filtrate again. If the latter is put on blood-agar and incubated the bacillus grows again. It is quite easy to give the disease to a susceptible animal using either the viral or bacillary forms. The dead virus phase has proved to be a feeble antigen, but the dead bacillus phase has proved to be most powerful for both prevention and cure.

Two slides (Figs. 6 to 13) show the isolation of the pure foot-and-mouth bacillus, its conversion into the virus form and reconversion into the bacillary form.

This next slide shows the growth of the swine fever bacillus from its virus form. I am quite sure that dead antigen will prevent and rapidly cure this disease.

The slides of rabies illustrating the production of the bacillus from "virus fixe" and its reconversion not only give us the expectation of a potent dead antigen for prophylaxis but also for the first time the chance of curing this terrible disease.

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These slides are particularly interesting to me. This work was a sequel to an investigation into the prevention and cure of fowl-pox with a dead bacillary antigen. A complete account of this work is in my book "The True Nature of Viruses." It was published also in the "Journal of Pathology" in 1924. All the early cases and 50 per cent of the advanced cases were cured. The disease was transmitted to healthy fowls by spraying the live bacillus on to the throats of healthy fowls. All the next year's stock were inoculated and not a single case occurred. What a contrast to the burning and slaughtering. One unfortunate owner had this experience three times in six years.

I was enabled to investigate in collaboration with Mr. David O'Brien, M.R.C.V.S., now a Veterinary Official in Tasmania, an outbreak of fowl paralysis. The causal bacillus was isolated from a swollen infected nerve and from the heart blood. An antigen was prepared and Mr. O'Brien was able to cure every affected fowl and prevent any other getting it. I have no doubt whatever that the serious problem of fowl pest can be solved.

At the same time there was an outbreak in London of smallpox with cases at Hendon and Dartford. From every case but one, I isolated a characteristic Gram-negative bacillus and later I recovered a similar organism from every case in an outbreak in King's Lynn. I produced a pox in a rabbit with this microbe. In the case of small-pox one has the safe resource of vaccinia for experimental purposes. One can grow the vaccinia bacillus from any specimen of lymph. I used to obtain it by infecting the mucous membrane of a rabbit's nose, but later I found that this was unnecessary for it would grow quite well on blood-agar. In search of an independent test I asked Monckton Copeman to do some experiments to see if he could not give rabbits a pox on their skin. He, the founder of the National Laboratories at Hendon, had several successful results (Figs 14 to 17).

The live bacillus was rubbed into the scarified skin and a scab formed; this scab was then rubbed on to a scarified area of another rabbit and two vesicles formed. The material from these vesicles was then rubbed into the scarified skin of another rabbit and a typical vaccinia was produced. This was the last communication I made to the Pathological Society and I said that I was of the opinion that these experiments demonstrated the absolute falseness of the virus theory. I found that if a rabbit was inoculated with a dead antigen of this bacillus it was impossible to infect it with the live virus. I have cured many cases which were ill following infection with the live virus. Unfortunately, many had their central nervous system permanently ruined. I am persuaded that if active immunisation with the dead bacillary antigen were used when the first symptoms of small-pox appear the disease could be cut short.

All systems of vaccination with living virus should be given up. The endothelial cells will let the dead microbial antigens through to the tissues but they will do all in their power to stop the live virus as you will see by this slide taken from an experiment by Adami long ago. You will see that an endothelial cell has become a macrophage to prevent the bacilli getting through to the liver cells. Dead microbes would have been allowed through.

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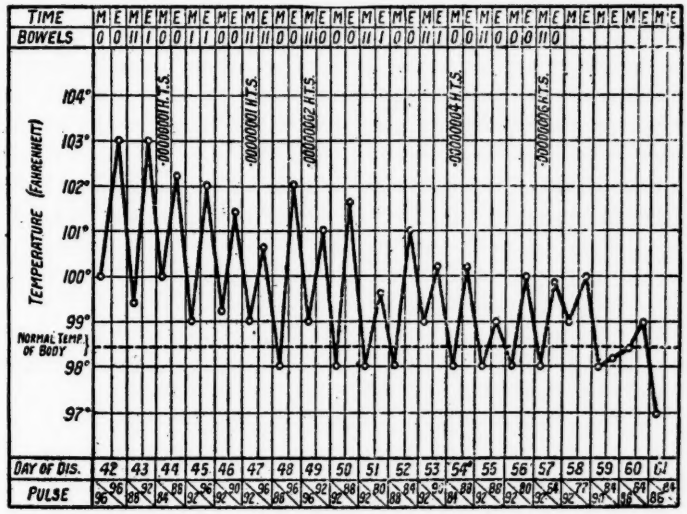
I should like to tell you something about another disease with a virus phase, viz. tuberculosis.

Usually you will not see any acid-fast bacilli either in pleural effusion or in the large pearly tumour-like masses. I will describe the case of a young woman who was delirious and suffering from acute lobar pneumonic phthisis. The case was considered to be quite hopeless. The woman made a brilliant recovery



ACUTE LOBAR TUBERCULOUS PNEUMONIA.

Fig. 18

A young woman with acute lobar pneumonic phthisis of the lower lobe of her right lung who had been delirious for over a fortnight treated with H.T.S. (human type tubercle-bacillus solution). This case made a perfect recovery with only a slight X-ray shadow in the centre of the lobe. She has remained perfectly well for over twenty years.

after immunisation with tuberculosis antigen H.T.S. Human-Type Tubercle bacillus solution. The solution is of acid-fast T.B. in benzoyl chloride. She was alive and well thirty years after treatment. Dr. Argenti has used the antigen for prophylaxis in calves that were going to be milch cows in Uruguay with 100 per cent results. I will read you a letter from Docteur Veterinaire Lours about his cure of a cow with advanced T.B. following the use of H.T.S.

(N.B.—In this part of France the milch cows are confined to the farmyards except for about a month on the stubbles in the autumn. Tuberculosis is rife.)

From Fernan Lours, Doctor of Veterinary Medicine,
La Ferte-Saint-Aubin (Poiret),
24.4.38.

My Dear Doctor,

It is with the greatest pleasure that I give you the results obtained with your antigen. On the whole lot treated there was a very marked improvement.

Shining coats, general state very good, milk doubled. The farmer on whose farm the trials have been made (M. Goyet, Fere du Duret) has said to me: "One would say that the beasts had been out on grass for a month."

But I call your attention especially to one beast completely at the end of its tether. An old cow having had many calves, I do not know how many, and only worthy of the knacker. It gave many positive reactions to the tuberculin test. It had most of the clinical signs of tuberculosis very marked. Well, following two injections of the antigen (H.T.S.) this cow which had its udder withered, which had ceased to eat, gave again 6 to 7 litres of milk, and regained 60 kilos of weight and a very good general condition. It is so well that it seems phenomenal and note well there was no shock, no reaction after the injections which might have been expected in this poor beast.

I cannot tell you more. If you would like to know what I really thought, it was, that I had given the injections of your antigen to a beast that they would kill. It is perhaps a little ruthless, but it is, I believe quite human to such to destroy a new thing by a check, saying to oneself, one has seen so much of vaccines and of these serums that claim to cure everything.

Note well I have arranged with M. Goyet that this cow will be killed at the abattoir of La Ferte in your presence so that you will see the state of the lesions, the evidence of which will still be present.

With my best remembrance, accept my warm sentiments

(*signed*) F. LOURS.

It was killed and found to be completely cured.

Similar recoveries in human beings have been produced for something over thirty-seven years. M. Lours and his fellows about Orleans had been equally startled and gratified by the dramatic recoveries of cases of foot-and-mouth disease.

Inoculation is safe and inexpensive. Since a very powerful, perfectly safe and very inexpensive antigen is available it is quite unjustifiable to use a dangerous live one like B.C.G.

I will show slides which Figs. 19 to 22 & 27 demonstrate the production of a Jensen Rat Sarcoma in the skin, with secondary tumours in the lungs, by injecting a minute quantity of a live gram positive bacillus into the skin. It is the virus phase of this bacillus which causes Sarcoma and you will note that exactly similar gram positive bacilli can be isolated from all sarcomata and also grown from the blood of leucæmias. The cause of all Carcinomata, with the exception of some of those affecting the ovary, are the virus phase of gram-positive cocci. The virus lives in the nucleus of the cancer cells. If this was not so then the tumour would not be malignant. There is no cancerous growth from which the causative microbe cannot be grown and growths have been made to disappear after immunisation with dead antigens of the microbic phase (Figs 28 to 30).

Well, Mr. President, that is all I have time to say to you this evening, I expect you think it quite enough. I have only been able to give this audience a glimpse of this great science. If I could get your help and co-operation in exploring the field further we might bring great benefit to the world.

Discussion

The discussion was opened by Dr. N. M. Larin.

Mr. President, Ladies and Gentlemen,

I feel you have done me a signal honour by asking me to open the discussion on the subject of Dr. Crofton's address to-day. On the other hand, I cannot help feeling that this honour is one of the most difficult tasks I have ever had during 25 years of my research work on bacteria and viruses.

Dr. Crofton's address was not concerned with the results of his experiments on the life cycle, either of one particular virus or one particular bacterium. Dr. Crofton spoke of rather a wide subject. This was, his hypothesis of transformation of animal viruses, in general, to bacteria and vice versa. Dr. Crofton spoke also on the subject of active immunity to virus diseases, and he claims that the difficult problems of active immunisation against virus diseases can be solved easily provided that "microbial antigens," to use his phrasing, are made into vaccines and injected into animals or human beings. Thirdly, Dr. Crofton stated that "microbic antigens" are the most "specific drugs" we have ever had for the treatment of acute and chronic infections.

You will undoubtedly remember those many thousand workers all over the world who are and have been engaged for many years on the various problems of viruses, bacteria, immunity and treatment of infectious diseases. Now it appears from Dr. Crofton's address that all this enormous amount of brain power contributed to ensure a comparatively modest progress of bacteriology and virology is spent in vain, for all these problems have been solved.

My difficulty is the very vastness of the subject of Dr. Crofton's address. This subject is so enormously large that my life time seems to be not sufficient even to approach experimentally a minute fraction of all these problems. Secondly, Dr. Crofton's ideas and his proofs are, in my opinion, a matter for a philosopher to discuss, and I must confess that during many years that I have been a bench research worker I have always felt that I was rather a poor philosopher. I say—that is a matter for the philosopher to discuss because Dr. Crofton's claims are not supported by convincing cast-iron proofs.

One might well say that any revolutionary idea in science has met a resistance and that the most usual argument of those opposing fresh and revolutionary ideas was that these ideas were not supported by convincing cast-iron proofs.

Still, if one remembers, for instance, the ideas and proofs of Pasteur, Lord Lister, Pavlov and other revolutionaries in science one would also recollect that there opponents had to admit that the proofs they opposed were quite tangible.

Dr. Crofton's "proofs" are of a different kind. His proofs are intangible, they being either his ideas or they are his own interpretations of certain established principles of bacteriology, virology and immunity.

Dr. Crofton considers that certain principles of bacteriology, virology and immunity taken in his interpretations are absolute and self-evident truth, while

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for others dealing with the same principles they appear only as a relative truth, that is to say that these principles being truth under certain set of circumstances only. This is more or less similar to the relativity of definitions of the East and West, or Saturday and Sunday. It is true that there is the East and West and there are Saturdays and Sundays, but this truth is a relative one because for people living for instance, in California, Japan and China are situated to the West rather than to the East. Also, you may remember that people who live near the Time Demarkation Line can have breakfast on Sunday morning and go after this for Saturday shopping. Having finished this, they can have a nice Sunday lunch and if desired, Saturday tea and Sunday supper. They can go to bed as they wish, either on Saturday or Sunday, this being on the same day.

For reasons of relativity I feel inclined to keep my feet on the solid ground of sober approach to Dr. Crofton's axiomatic principle—"microbic antigens" which is used by him as a foundation for building proofs for his fascinating ideas. Let us try to draw the curtain of mystery off these "microbic antigens" in order to see as to whether all antigenically active constituents of a bacteria cell can protect animals against bacteria concerned.

As a preface of this discussion I would firstly like to draw your attention to the tubercle bacillus which possess many different antigens to which immunologic responses, antibodies and hypersensitivity appear. It has been established, however, that neither of these antigens is the factor responsible for inducing specific resistance. We also know that the tubercle bacilli killed by various means fail to induce immunity, yet they provoke the formation of antibodies.

It may be that an antigen or antigens of the tubercle bacillus which are concerned in inducing protection, become so altered in the process of killing the cells as to be ineffective. Or there may be a quantitative factor involved, since living bacilli proliferate *in vivo,* while in the case of dead organisms the quantity of any antigenic substance is restricted to the amount injected. Finally, there may be an antigenic constitute of the tubercle bacillus growing in the tissues only and which is absent in the cultures *in vitro.*

In the case of the diphtheria bacillus, we find that the chief protection-inducing factor is exotoxin and the resistance consists of a direct attack by the host upon this. There is, however, evidence suggesting that another system of parasite-host interplay may also exist; that some component of the bacterial body itself may incite an antibacterial resistance and that a factor of the bacterial cell may account also for its capacity to become established in the host. Possibly a surface antigen may be the immunity-inducing factor and you know that type specific surface antigens have been amply described.

In gas-gangrene which is caused by one or by a combination of the toxigenic species of Clostridium, it seems clear that the toxins of these organisms are the substance of importance in accounting for acquired resistance to the disease. The toxins of the Clostridia are now fairly well documented; fifteen substances are listed as products of Clostridium perfringens alone. As for the possibility that a bacterial component may also be concerned in protective immunisation, there is little evidence to support this.

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In anthrax, the evidence at hand suggests quite clearly the existence of different antigens and also that acquired immunity to anthrax may be implemented in at least two different ways depending upon the responding host. It has been established that relationships between immunising factors of the bacilli and a host's response are different, for instance, in rabbit type of host if compared with mouse type of host.

It can be inferred from this evidence that bacteria possess many antigenic components of which only a very few are protection-inducing substances. It is also clear that the active protection of different animal species against the same culture of bacteria might be induced with different antigenic components and be developed by different ways. In connection with this it is not clear at all what kind of bacterial components in Dr. Crofton's opinion induce active protection against virus diseases and particularly, it is not clear which bacterial components are called by Dr. Crofton "microbic antigens."

Mr. E. O. Longley said that whatever might be thought of Dr. Crofton's bacteriological theories, their practical application apparently produced impressive clinical reults. There was independent evidence of this, and in the last analysis it was not theories but results which mattered. At the same time he considered it desirable to face the broad principles involved in Dr. Crofton's ideas, as reflected in the following questions: are the viruses essentially and invariably ultra-microscopic and never cultivable on artificial media; and are the bacteria always and essentially visible and invariably cultivable on artificial media? While the answers to these questions might be considered, by definition, in the affirmative, these attributes conventionally defining the bacteria and the viruses respectively, the question arising in important corollary was, can it be considered as established beyond all possible doubt that no virus can ever mutate to a bacterial form, and no bacterium ever mutate to a virus phase? He submitted that the possibility of such mutation had never been excluded beyond all shadow of doubt. His own experience abroad, working on the so-called virus of caprine pleuro-pneumonia was not irrelevant to the case. When in India, he had tackled this research problem as though the disease were in fact of virus etiology, in accordance with the text-book and other literature. The agent was filtrable, and like all previous workers he failed completely to isolate in culture, on any of an extravagant variety of media, any organism that could be ascribed an etiological role. But an examination by dark-ground microscopy of pathogenic material disclosed that all such fields were swarming with organisms of the characteristics and distinctive pleomorphic morphology of the pleuropneumonia group. Failure to invoke this optical technique by previous workers during all of the 80 years that the disease had been the object of study, had led to the entirely erroneous conclusion that the disease was of virus origin. In subsequent studies in Nigeria, where he had been appointed to continue this work, and employing as culture-medium pure unadulterated goat serum incubated at goat's body temperature, he had succeeded in cultivating the organism, which had a growth so tenuous and slow that it might readily be overlooked. The precise source and freshness of the inoculum were found to be of supreme importance. The obvious inference was

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that the long history of negative cultivation was due to these subtle differences in technique, though for all that he could say the Indian strain might not be cultivable at all. At all events, with cultivation thus achieved Koch's postulates could be and were satisfied, and in fact the organism was confirmed by the Lister Institute, London, to be a new member of the pleuropneumonia group, now lodged in the National Collection of Type Cultures. The point was that here was a disease of, so to speak, approved virus etiology, due in fact to an agent filtrable and visible, pleomorphic and gram-negative, and cultivable or otherwise according to the subtleties of procedure. Such a history at least did nothing to discredit Dr. Crofton's ideas. He noticed that Dr. Crofton emphasised the supreme importance in his work of the autogenous source and freshness of all inocula, likewise of all media, and that he insisted on the use of only primary growths and first subcultures for the preparation of the antigen, further subcultures being antigenically unreliable. Such stipulations were unquestionably sound bacteriologically, and he wondered how much of Dr. Crofton's clinical success depended on his rigorous observance of these principles, which tended in these days of mass-produced vaccines and antibiotic therapy to be rather neglected. Some time ago he had had the pleasure of lunching with Sir Thomas Dalling and Dr. Crofton, and two remarks passed by Sir Thomas in discussing Dr. Crofton's ideas were of contextual interest. Sir Thomas had remarked in discussing distemper, and the role of Ferry's bacillus (bronchisepticus), that until the advent of the Laidlaw-Dunkin work he himself had been "a confirmed Ferry-fan." Sir Thomas also described how, when working on infectious ophthalmia of horses, he had been puzzled consistently to isolate a gram-negative pleomorphic organism, to which he could ascribe no satisfactory role.

Miss McLelland said she was not a virologist but a clinician and she found Dr. Crofton's methods had worked in practice. Dr. Crofton had recently made her a vaccine from a case of Canine Viral Hepatitis; the dog had died and post-mortem and laboratory examinations of liver and gall bladder had confirmed the *in vivo* diagnosis. The vaccine was made from the urine. Several cases showing clinical symptoms of hepatitis had recovered quickly when this vaccine was administered. One had to experiment with vaccines, each case having to be watched carefully; in some acute cases vaccine must be given every 24 hours for two to three days and then, if the response is good, a longer time may elapse between injections which should be continued until all symptoms have disappeared. In chronic cases one must be very careful, often one injection a week being sufficient, otherwise the animal might have reactions following the injections; the reaction taking the form of a severe attack of the original symptoms.

Mr. Bateman said that first he would like to say that he was quite sure that if Sir Thomas Dalling were here to-night he would claim to be pro-virus and not pro-Ferry!

In his early years in practice the up-to-date method of trying to prevent or cure distemper was by means of killed bacterial cultures, and probably the most widely used was the product marketed under the name of Canine Distemper Mixed Bacterin. This was described as "A polyvalent bacterin each c.c. of which

contains: B. bronchi-septicus (canine) 3,000 million: Staphylococcus albus and aureus (canine) of each 300 million: Streptococcus canine 400 million, sterile and ready for immediate use, for the prevention and treatment of canine distemper, etc."

Three graduated doses were given for prophylaxis, larger doses were given for treatment.

This treatment was based on the work of Ferry and Macgowan on Bronchisepticus, but those of us who used the product in general practice—enthusiastically at first—were forced, reluctantly, to the conclusion that it was not efficacious. It did not prevent distemper and it did not cure it.

It was because of the complete lack of protection given by this excellently presented material and other killed bacterial substances that the work of Laidlaw and Dunkin was instituted and sponsored by "The Field." One reads and hears of many unworthy attacks made on the works of these two gentlemen, but the facts stand unchallengeable that they produced a method which, despite its failings, when it came to be marketed on a large scale, did protect dogs against distemper and for the first time, in this country, in his (Mr. Bateman's) lifetime.

Laidlaw and Dunkin did an extraordinarily good job for the canine species of the world. This did not mean that all other workers should cease to try to find other methods. For his own part he welcomed the lone research worker, and he included Dr. Crofton among them, but he would most earnestly plead that due regard should be given to the line of thought and work of others even if their work does not seem to agree with one's own.

His experience of Dr. Crofton's methods in distemper was limited, but he recounted it as far as it goes:—

In 1954 he decided to give the Crofton method a chance in the treatment of dogs which were trickling into his hospital, some with distemper. Before he went into details of the cases he said that the first thing to interest him was the speed at which the autogenous vaccines were prepared. For example, three throat swabs were taken one night, delivered by hand to the laboratories the following morning at 11 o'clock and the completed three sets of autogenous vaccine were made ready, packed and put on the 3.45 p.m. train on the following day. All were marked exactly alike, "Gram neg. bacilli," and each set contained 12 doses graded from an initial dose of $\frac{1}{4}+$ to a final dose of 12+ million.

In all he took nine swabs and he detailed his cases below. All were young dogs of unknown distemper history, except No. 5, believed to be immune.

Swab No. 1. Report Gram neg. bacilli. A dog with high temperature and tonsillitis. Vaccine arrived 48 hours after onset. Temperature had dropped from 105° to normal in the first 24 hours. Vaccine not used. Temperature normal for 17 days dog sent out.

Swab No. 2. Report Gram neg. bacilli. A bitch with initial temperature of 103.8° dropped to normal for 12 days and on the 13th day—the day on which secondary symptoms might be expected to develop if the case was distemper, he took the throat-swab. The gram negative vaccine arrived but was

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not used. There was no further temperature rise and the bitch was discharged from hospital—21 days normal.

Swab No. 3. Report Gram neg. bacilli. This was from a dog admitted with tonsillitis and a temperature of 104.8°. Temperature dropped in 24 hours and remained normal until the fifth day, when it rose to 101.6° and swab was taken. Temperature fell again on the sixth day and on the eighth day the first dose of gram negative vaccine was given. On the 9th day the temperature rose to 101.8-102° and dropped next day. Four doses vaccine were given and there was no further temperature rise.

Swab No. 4. Report Gram neg. bacilli. A dog admitted to hospital with a nasal discharge. He had been ill for 24 hours ten days previously—a typical clinical distemper case. Swab was taken on fourth day, treatment started on eighth day. He had the complete course of 12 injections lasting until the 53rd day, when he still continued to discharge. His temperature was finally normal on the 73rd day and he was discharged, cured, on the 85th day.

Swab No. 5. Report Gram neg. bacilli. Temperature 105°. Swab taken second day. Treatment started on sixth day—two days after he had returned to normal. He received five injections lasting until the 24th day and was discharged having been normal 22 days.

Swab No. 6. Report Gram neg. bacilli. Admitted with temperature of 103.4°. Swab taken seventh day. Dog took pneumonia 10th day. Vaccine not used. Case ran a typical distemper course with nasal discharge, diarrhœa, etc., and was finally sent out recovered on the 40th day.

Swab No. 7. Report no growth. A typical distemper case admitted in the interphasic period. Nasal discharge 10th day. Swab taken 11th day. A "stock" vaccine was sent, six doses. These were injected at intervals until the 32nd day. Discharge continued until the 49th day and the animal was finally sent out, recovered, on the 55th day.

Swab No. 8. Report Gram neg. and cocci. Tonsillitis, eye discharge, temperature 105°. Put on Swab No. 2 vaccine at once and received the first seven doses. No improvement. Swab sent 24th day and the new treatment commenced on 28th day. The dog received eight doses but died very weak and with hard pads on the 58th day, 30 days after autogenous vaccine had been started.

Swab No. 9. Report Gram. neg. bacilli. Kennel mate to No. 8. A typical distemper case. Swab taken 19th day. Vaccine not used. Dog left hospital, cured, on 53rd day.

Unused vaccines were employed on two typical distemper cases. Both dogs lived but there was nothing in the result to indicate that the course had been altered or shortened.

One dog (Swab 1 Vaccine) had recovered completely in 44 days, whereas the other (Swab 9 Vaccine) was left with a slight loss of co-ordination after 48 days.

Remarks. One would have appreciated the identification of the gram negative bacillus on each batch of vaccine. Each report had stated "Gram negative bacilli." Apparently a gram negative bacillus was present in every throat swab and since the vaccine was made from it, it must be assumed to have some significance in the laboratory. Not all the dogs had distemper. One knows from experience that distemper in dogs living in large kennels does not fade out without complication, after the initial temperature rise.

A survey of the results of the use of autogenous vaccine—as far as one could judge from the few cases examined—would seem to indicate:—

(*a*) That the general course of the disease in typical distemper was in no way shortened, e.g., No. 4.

(*b*) Similar cases, untreated, recovered in as short a time as treated cases.

(*c*) The vaccine had no effect on the one hard-pad type of case, No. 8.

It has been stated to-night that autogenous vaccination is no longer used, but surely that is because we found that, in distemper, when it was used, it failed to protect our dogs.

Mr. Halpin said that despite the opposition of the orthodox pathologists, Dr. Crofton had stuck to his theory. But had it ever been given a fair and unbiased trial? Many proven cures of the past had been regarded as revolutionary at their inception, and whether or not his theory was correct, scientific probity demanded for Dr. Crofton a full and thorough investigation under the most stringent conditions.

Dr. Culver Evans spoke in support of Dr. Crofton's theories. She had been interested from her student days in Immunology and for the last ten years she had been closely associated with Dr. Crofton's work. In human pathology it worked as well as it appeared to work in the veterinary world. She would like to instance two cases, one, just after the war, of severe septic abortion. She was associated with Dame Louise McIlroy in the treatment of the case and Dr. Crofton was called in and the woman recovered with dramatic swiftness and had since conceived. That was a very great tribute. The second was her son, who 13 months ago contracted bulbar poliomyelitis. Before a clinical diagnosis was made, Dr. Crofton got a typical growth of the organism associated with poliomyelitis. Dr. Crofton made an antigen and she administered it. She was told by the ward sister that every time a dose was given the patient improved clinically but it was not maintained. She explained that improvement could only be maintained when sufficient antibodies had been produced, which would be provoked by gradually increasing doses of the antigen. After the ninth attack of respiratory failure with unconsciousness and Cheyne-Stokes breathing with marked cyanosis, cardiac involvement and complete exhaustion, she gave him a dose. She waited half an hour and then saw the night sister, who had just come on duty, to ask how he was. The sister said: "I don't know what you are worried about, he is *much* better." That was all she wanted to know—the antigen had worked. He improved steadily thereafter and three months later he was

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back at his work as a general practitioner. These were two examples of what she had seen clinically, and clinically she was convinced that Dr. Crofton's theory was correct. It did work and it was simple. Hitherto bacteriologists, while admitting the theoretical efficacy of a specific antigen, had not found a way of producing it so that it was effective when given to the patient. Dr. Crofton had found a way of producing an effective and specific antigen for a wide range of infectious diseases.

Dr. Crofton felt that the only criticism he had to meet was that of Mr. Bateman. Mr. Bateman had got Dr. Crofton to make a series of autogenous antigens for dogs and then had apparently not given the dogs their own specific antigen, but in one or more cases had used them for other dogs without ascertaining their bacteriology. Mr. Bateman seemed to be very surprised that in every case but one Dr. Crofton reported the presence of a gram negative bacillus. Dr. Crofton asked Mr. Bateman how he knew a dog had distemper, for really it was quite impossible to know that a dog had distemper without ascertaining whether or not the dog had the specific microbe, the gram negative bacillus of Copeman. Dr. Crofton related how he had asked Dr. Andrewes how he knew that his colleague, Wilson Smith, from whom he had isolated an influenza virus, had in fact influenza. He could only know that Dr. Wilson Smith had influenza by the fact that his throat and nose discharges were swarming with the gram negative bacillus of Pfeiffer and he (W.M.C.) pointed out that if Dr. Andrewes had in fact given his ferrets influenza with the filtered material he could not have failed to isolate the influenza bacillus from the ferrets' nasal discharges. This had not been attempted. Mr. Halpin had asked him, had there not been a proper investigation of all this work? Dr. Crofton replied: "Nothing had been left undone to suppress this work, and nothing was being left undone at this moment to suppress this work."

Dr. Bell, on behalf of the meeting, thanked Dr. Crofton for giving them an interesting account of his theories.

STUDIES ON THE PIG LUNGWORM (*METASTRONGYLUS* SPP.)

II—Experimental Infection of Pigs with *M. Apri*

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Introduction

As part of a study of the pig lungworm (*Metastrongylus* spp.) which began in 1952, a series of experiments with pigs was carried out using *Metastrongylus apri,* and the results form the subject of the present paper. Methods used for earthworm culture (Dunn, 1954), the incidence and features of natural infection of pigs (Dunn, Gentles and White, 1955), and the results of experimental infections of guinea pigs (Dunn, in preparation) are dealt with elsewhere.

Several workers have carried out experimental infections in pigs in the course of attempts to elucidate the life history of the pig lungworm. Thus Hobmaier and Hobmaier (1929) state that a pig dosed with infected earthworms had to be slaughtered 15 days later because of verminous pneumonia. Schwartz and Alicata (1934), who studied the development of lungworms in 22 pigs killed at intervals ranging from two days to six months after eating infected earthworms, did not record any marked ill effects in the pigs, and attributed their absence to the light or moderate doses of infective larvæ used. The lesions they describe include petechial hæmorrhages in recent infections, and bronchitis, pneumonia and emphysema later. Von Schuckmann and Zunker (1931) also do not mention the occurrence of symptoms in the pigs they used, but Matoff (1936) refers to the death of a young pig 24 days after it had been fed infective larvæ. All these workers were concerned mainly with the parasite itself, rather than with its effect on the definitive host. There appears to be no reference relating to experimental work designed to show the effect of the parasite on the definitive host.

Material and Methods

Animals. A preliminary experiment was carried out on three weaner pigs from two different litters which had been on pasture before weaning. The main experiment was conducted with a litter of seven pigs farrowed from a sow which had been kept at the laboratory in a concrete pen for two months previous to farrowing. The laboratory-bred pigs were never allowed out of their pen and had no contact with naturally infected earthworms. The pigs were given creep feed *ad lib.* and from two weeks to weaning they also received, three times a week, sods of turf steam sterilised for two hours. After weaning they were fed wet weaner and fattening meal and were given well-washed cabbage daily. The pigs were weighed weekly and fæces samples were collected from them in the weighing crate, as required.

Infected earthworms. Eisenia fœtida, which is the common earthworm of manure and compost and the species most easily cultured in the present study and most easily infected with lungworm larvæ, was used to infect the pigs.

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The method of culture, and of preparing and infecting earthworms with *Metastrongylus apri,* has already been described (Dunn, 1955).

The dose of larvæ given to a pig was estimated by counting the larvæ in worms (five to ten) picked at random from the culture. The larvæ were counted in the anterior end of the worm which was pressed between two slides. The average number of larvæ per worm was then calculated and the approximate dose estimated for each pig. For unweaned pigs, the anterior ends of as many worms as were required were chopped up and enclosed in gelatine and fed as a bolus placed on the back of the tongue. For older pigs, chopped whole worms were fed in wet meal or milk before the morning feed.

Fæcal examination. Fæces samples from individual pigs, including the sow, were examined weekly, except in the preliminary experiment when daily samples were examined. For the first five weeks of the preliminary experiment, fæces were examined by the McMaster flotation method and also by centrifugation with saturated sodium chloride solution. It was then found that saturated sodium chloride was unreliable for detecting lungworm eggs and that saturated magnesium sulphate solution ($MgSO_4$, 7 H_2O. S.G. 1.285) was much more effective (Dunn and White, 1954), and this technique was used in all subsequent fæcal examinations.

Hæmatology. Differential leucocyte counts were made weekly. Blood was taken from an ear vein when the pig was in the weighing crate, and films were stained immediately by Leishman's stain. Counts of 200 leucocytes on each film were made.

Autopsy. Two pigs, P1 from the preliminary experiment and M1 from the main experiment, were slaughtered at the laboratory a few days after receiving infected earthworms, and a detailed autopsy was carried out. All the other pigs were sent to a bacon factory at 6 to 7 months old, except for one which was underweight and was sold to a butcher. The lungs and liver were in all cases brought back to the laboratory and examined macroscopically and histologically and any parasites present were counted.

Results

TABLE 1 Details of Experimental Pigs

Pig No.	Larval dosage				Coughing, presence or absence	Fæcal egg counts		Eosinophils		Age at slaughter (wks.)	Wt. at slaughter (lbs.)	Lungworms at slaughter	
	Total	No. of doses	Age at first dose (wks.)	Age at last dose (wks.)		First appearance (days)	Peak count (e.p.g.)	Peak (%)	Time of peak (wks.)			♂	♀
P1	3,500	2	10	10½	+ +	-	-	-	-	10½	41	(natural inf.) 93	
P2	3,000	1	10	-	+ +	28	733	-	-	27	206	7	15
P3	CONTROL	-	-	-	-	-	-	-	-	28	205	0	0
M1	4,100	2	2	8	+	28	3,100	10	2	8	43	86 (left lung)	600
M2	3,100	2	13	17	-	49 (21)*	7	11	2	27	200	3	10
M3	8,000	16	8	23	+	21	79.5	15	3	27	180	5	22
M4	5,000	3	2	2½	+ +	28	7,750	14	2	27	220	490	998
M5	20,000	2	12	18	+	21	31	16	2	27	197	0	1**
M6	500	1	18	-	+	20	50.5	14	2	27	188	17	4
M7	CONTROL	-	-	-	-	-	-	2.5 (range)	-	27	159	0	0

* 49 days after initial small dose (100 larvae), 21 days after large dose (3,000 larvae)

** Immature lungworms also in sections

A. Preliminary Experiment (3 pigs).

Pig P1. This was the runt of a litter of nine farrowed at the laboratory. At the age of 10 weeks it was given an initial single dose of 3,000 larvæ, and a second dose of 500 larvæ three days later. It was killed five days after the initial dose. The only macroscopic signs of recent larval migration were hæmorrhages in the lungs; one histological section of such a lesion showed a larva surrounded by eosinophils. There was also a necrotic focus surrounded by eosinophils in a section of a mesenteric lymph node which might have been the result of larval migration. The small intestine contained 26 *Ascaris lumbricoides* and the lungs contained 93 mature *Metastrongylus apri*. Daily fæcal examinations using saturated NaCl had shown *Ascaris* eggs but *NO* lungworm eggs, but the adult lungworms found at autopsy were clearly the result of a natural infestation which was later found to be present in each of the remaining eight pigs of this litter when magnesium sulphate solution was used instead of sodium chloride.

Pig P2. This was one of a litter of eight weaners bought from a farm in the Wirral district of Cheshire. When 10 weeks old it was given a single dose of 3,000 larvæ. It began to cough two days later and coughing increased to a maximum three to four weeks after dosing and then subsided. Weight gains were satisfactory and at 27 weeks old it weighed 206 lb. and was classified as Grade A after slaughter at a bacon factory.

The lungs showed lobular areas of emphysema and numerous greyish nodules in the diaphragmatic lobes. A total of 22 adult *M. apri* (15 females, seven males) was found. Fæcal examination of the pig during life had shown the eggs of *Œsophagostomum* or *Hyostrongylus* spp. and *Trichuris* spp. at all times. Lungworm eggs were first detected (1 e.p.g.) 28 days after the initial dose of larvæ had been given. Their number rose to 300 e.p.g. by the 38th day and ranged between 100 and 733 e.p.g. in daily samples taken over the next ten weeks. The level then fell slowly until, during the five weeks before slaughter, it was always below 5 e.p.g. The viability of the eggs was shown by successful infection of earthworms with the infected fæces.

The eosinophil counts reached 7 to 10 per cent between the 10th and 28th days after dosing with infective larvæ.

Pig P3. This was a litter mate of P2 and served as a control. At no time was it observed to cough and it made fair weight gains until it was killed for bacon at 205 lb. (28 weeks) and was classed as lean. The lungs were normal and contained no lungworms. The stomach contained *Hyostrongylus rubidus*, and the intestine *Œsophagostomum dentatum*. Fæces examinations had always shown the eggs of these two species, but lungworm eggs were never found. Eosinophil counts were nearly always within the normal range (4 ± 2 per cent) quoted by Holman (Boddie, 1954), and at no time exceeded 7 per cent.

B. Main Experiment (7 pigs).

During the seven weeks prior to farrowing and the week following farrowing the fæces of the sow were examined weekly. An occasional egg of both *Œsophagostomum* spp. and *Trichuris* spp. was found, but never more than

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1 e.p.g. No *Ascaris* or lungworm eggs were found at any time. The almost complete freedom of the sow from helminths and the daily removal of manure meant that the young pigs were not exposed to infective helminth eggs or larvæ other than those deliberately fed to them, and in fact none of the litter ever showed any helminth eggs in the fæces during life other than the lungworm eggs which resulted from experimental infection.

Pig M1. At two weeks this pig was fed 3,500 larvæ. It received a second dose of 600 larvæ six weeks later and was killed 48 hours afterwards. Slight coughing was observed two weeks after the first infection. Weight gain was satisfactory. At autopsy both lungs showed extensive areas of consolidation, and lobular areas of emphysema. The left lung yielded 686 adult worms (600 ♀ 86 ♂). The right lung was kept intact as a specimen. Histological sections showed an extensive verminous bronchopneumonia with multinucleate giant cell formation around the lungworm eggs lying free in the alveoli (Fig. 3) and a marked bronchitis with epithelial proliferation, mucoid metaplasia and peribronchial lymphoid hyperplasia (Figs. 5 and 6). The liver showed several "milk spot" lesions (Fig. 4) with the gross and histological features of those caused by *Ascaris* migration (Oldham and White, 1944). As no *Ascaris* eggs were found at any time in the fæces of the sow or her litter, the "milk spot" lesions were probably due to accidental migration of lungworm larvæ through the liver. This was reported by Schwartz and Alicata (1934), who found lungworm larvæ in the liver of experimentally infected pigs and guinea pigs. The serosal covering of the cæcum showed a number of grey nodules about 1 mm. in diameter which were seen in sections to consist of degenerating neutrophil and eosinophil leucocytes surrounded by a fibrous capsule. They may have been produced by migrating lungworm larvæ, since it is through the wall of the cæcum and colon that they enter the lymph stream (Schwartz and Alicata, 1934). A few lungworm larvæ were found in fresh press preparations of the cæcal lymph nodes.

The first egg count was made four weeks after the first dose of larvæ was given, and revealed lungworm eggs (83.5 e.p.g.). The count rose steeply to a maximum of 3,100 e.p.g. within a fortnight and was 574 e.p.g. when the pig was given the second dose of larvæ, two days before slaughter.

The eosinophil count rose to a peak of 10 per cent a fortnight after the first dose of larvæ was given, and then fell again and did not subsequently rise above 3 per cent.

Pig M2. At 13 weeks this pig was given a small dose of larvæ (100) and then four weeks later a large dose of 3,000. No clinical symptoms were observed at any time, and the animal made good weight gains until it was slaughtered at 27 weeks, and the carcase was graded A (see Table 1). Each lung showed about 20 firm greyish nodules 2 to 8 mm. in diameter, mostly near the inferior border of the diaphragmatic lobes (Fig. 1), although a few were located in the cardiac and apical lobes. Occasional pale lobular areas of emphysema were seen near the posterior tip of the diaphragmatic lobes. There was no pneumonia. The left lung contained six adult lungworms (5 ♀ 1 ♂) and the right lung contained seven worms (5 ♀ 2 ♂). The liver showed a few "milk spots." Sections of the

lungs showed areas of peribronchial lymphoid hyperplasia (Fig. 7), together with hypertrophy of the muscle coat of associated bronchioles, some of which contained degenerating adult worms.

Lungworm eggs first appeared in the fæces seven weeks after the first dose of larvæ and three weeks after the second dose of larvæ was given. The counts rose the next week to 7 e.p.g. and then fell again, and did not exceed 3.5 e.p.g. up to the time the pig was slaughtered at 27 weeks of age.

The eosinophil count fluctuated widely from week to week; the highest values, 11 per cent and 9 per cent, occurred two and six weeks respectively after the large dose of larvæ.

Pig M3. This animal was fed a total of 8,000 larvæ in the form of 16 weekly doses of 500 larvæ each from the age of 8 weeks. Coughing was noticed three weeks after the first dose, but not afterwards.

Weight gains were poor after the age of 12 weeks and the pig had failed to reach bacon weight when slaughtered at 27 weeks, four weeks after the last dose of larvæ. The lungs showed nodules and lobular areas of emphysema which were distributed as in pig M2. A total of 27 lungworms was found, 21 in the right lung (17 ♀ 4 ♂) and six in the left lung (5 ♀ 1 ♂). A number of worms were immature, although the last dose of larvæ had been given a month previously. Sections showed similar changes to those in M2 but fewer parasites were seen, although hypertrophy of the muscle of the bronchioles was especially marked.

Lungworm eggs first appeared in the fæces three weeks after the first dose of larvæ, rose to 79.5 e.p.g. three weeks later, and then fell rapidly to 1 to 3 e.p.g. until slaughter. The last two weekly counts before slaughter were negative. The eosinophil count rose to 8 per cent a fortnight after the first weekly dose of larvæ, reached 15 per cent a week later, and then varied between 3 and 11 per cent up to slaughter.

Pig M4. At the age of two weeks this pig was given a total of 5,000 larvæ in three doses over a period of four days. Coughing began two weeks after dosing and continued up to the time of slaughter. Weight gains were good and the eventual weight at slaughter was the highest of the litter and the pig was graded A. The lungs showed lobular areas of emphysema and consolidation which were distributed mainly along the inferior border of the diaphragmatic lobe. There were no nodules. The consolidated areas showed chronic inflammatory changes and lymphoid hyperplasia, which was diffuse rather than nodular. A total of 1,488 lungworms was recovered from the lungs at autopsy, 723 from the right lung (495 ♀ 228 ♂) and 765 from the left (503 ♀ 262 ♂). The worms were distributed throughout the whole of both lungs, but most of them were present in areas of lung which showed no macroscopic changes other than some chronic bronchitis. Lungworm eggs appeared in the fæces within a month of dosing, rose rapidly to 7,750 e.p.g. six weeks after infection, then settled down to a range of 200 to 600 e.p.g. in the last three months before slaughter.

The eosinophil count rose to 14 per cent a fortnight after dosing, and then subsequently failed to rise above 7 per cent. The mean value for the month before slaughter was 1 per cent.

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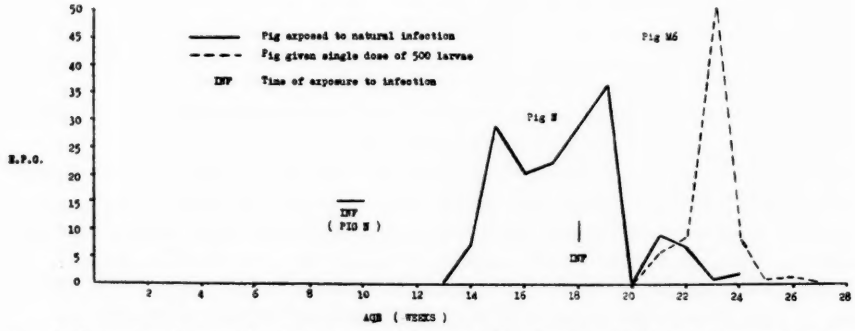
Pig. M5. This pig was given two doses of 10,000 larvæ each at 12 and 18 weeks of age. Despite these large doses, the only clinical symptom was slight coughing, which was noticed three to five weeks after the first dose of larvæ was given. Weight gains were good and the pig reached bacon weight in 27 weeks and was graded A. The lungs showed nodules and emphysema, as in pig M2, and a very marked hypertrophy of the muscle of bronchioles (Fig. 8). Only one lungworm was recovered, a female, in the left lung, but several immature worms were seen in histological sections (Fig. 8) occupying the lumen of the greatly thickened bronchioles. The liver showed "milk spots." Lungworm eggs (2.5 e.p.g.) were first found three weeks after the first dose of larvæ, then rose slowly over a period of seven weeks to 31 e.p.g., four weeks after the second dose of larvæ, and finally fell slowly to reach 0.5 e.p.g. at the time of slaughter.

The eosinophil count rose to 11 per cent and 16 per cent one and two weeks respectively after the first dose of larvæ was given. There was a second peak of 15 per cent six weeks later, and the count fell to within the normal range.

Pig M6. At 18 weeks of age this pig was given 500 larvæ. Until this time it had served as an uninfected control and had shown no eggs in the fæces, and normal eosinophil counts. Weight gains after dosing were about average for the litter, but the pig did not reach bacon weight at 27 weeks. The lungs showed nodules and areas of emphysema, together with small patches of consolidation at the free edge of both cardiac lobes. A total of 21 lungworms was recovered, nine from the right lung (2 ♀ 7 ♂) and 12 from the left lung (2 ♀ 10 ♂). All the lungworms except one were in the diaphragmatic lobes and were associated with the macroscopic lesions of lymphoid nodules and vesicular emphysema.

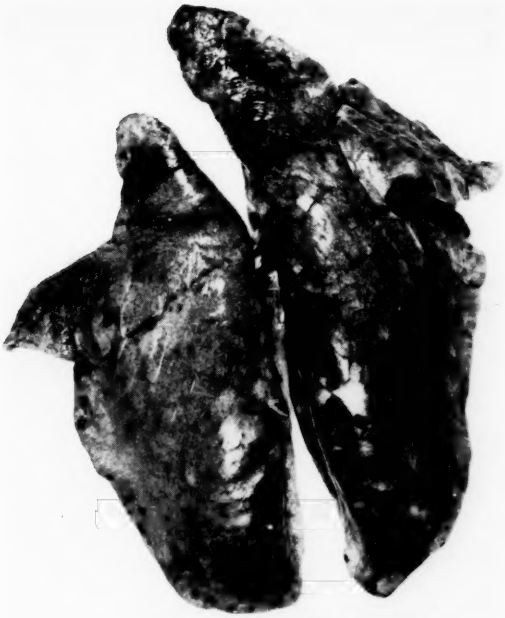
Lungworm eggs first appeared in the fæces 20 days after dosing, reached a peak of 50.5 e.p.g. after a fortnight (Fig. 9), and then fell to 0.5 e.p.g. two

Fig. 9 Comparison of egg counts of Pig M6 given a single dose of 500 larvae at 18 weeks with those of Pig H exposed to natural infection for one week just before weaning



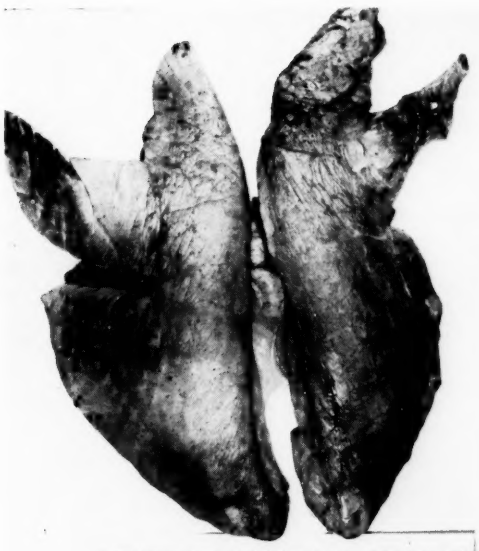
weeks later, and remained at about this level (the limit which was detectable by the $MgSO_4$ flotation method employed with 2 g. fæces samples) until the animal was slaughtered.

PLATE I



LUNGS OF EXPERIMENTALLY INFECTED PIG

Fig. I



LUNGS OF NATURALLY INFECTED PIG

Fig. II

(Article by Dunn, page 327)

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LEGENDS TO ILLUSTRATIONS

Fig. 1

Lungs of pig M2, killed at 27 weeks and given 3,000 and 100 larvæ 10 and 14 weeks previously, respectively. Typical lungworm nodules are seen, mainly along the inferior border of the diaphragmatic lobes. There is also a lobular area of vesicular emphysema at the caudal tip of the right diaphragmatic lobe. The small dark foci are hæmorrhages associated with slaughter. x⅓.

Fig. 2

Lungs of naturally infected pig at bacon weight showing nodules like those in Fig. 1 in all lobes. x⅓.

Fig. 3

Verminous pneumonia showing multinucleate giant cell formation around an embryonated lungworm egg (top left), and a free first-stage larva surrounded by eosinophils (bottom right). Pig M1, killed six weeks after receiving 3,500 lungworm larvæ. H.E. x 109.

Fig. 4

Liver showing focal interstitial eosinophil hepatitis ("milk spot") in same pig as Fig. 3. Lesions identical with those caused by migrating larvæ of *Ascaris lumbricoides* but no Ascaris found in sow or litter. H.E. x 116.

Fig. 5

Small bronchus containing an adult lungworm embedded in mucous exudate, verminous pneumonia, and early peribronchial lymphoid hyperplasia. Same animal as Fig 3. H.E. x 37.

Fig. 6

Medium bronchus of pig M1 (see Fig. 3) containing a mass of adult lungworms of both sexes and showing cellular infiltration of the bronchial wall and proliferation of the epithelium with papilliform projections less marked than in Fig. 5. H.E. x 42.

Fig. 7

Section through a typical lungworm nodule of the kind found in long-standing infection (pig M2, for details see legend to Fig. 1). The nodule consists of lymphoid tissue which has developed around bronchioles containing adult worms. There is some hypertrophy of the bronchiolar muscle. H.E. x 37.

Fig. 8

The marked hypertrophy of the bronchiolar musculature which is typical of chronic lungworm infection. The hypertrophy is in this case not uniform, and the lumen contains three still immature and stunted adult parasites. There is a marked hyperplasia of peribronchiolar lymphoid tissue, with follicle development as in Figs. 5 and 7. Pig M5, given 10,000 lungworm larvæ at 12 and at 18 weeks and killed at 27 weeks. H.E. x 37.

(*Article by Dunn, page 327*)

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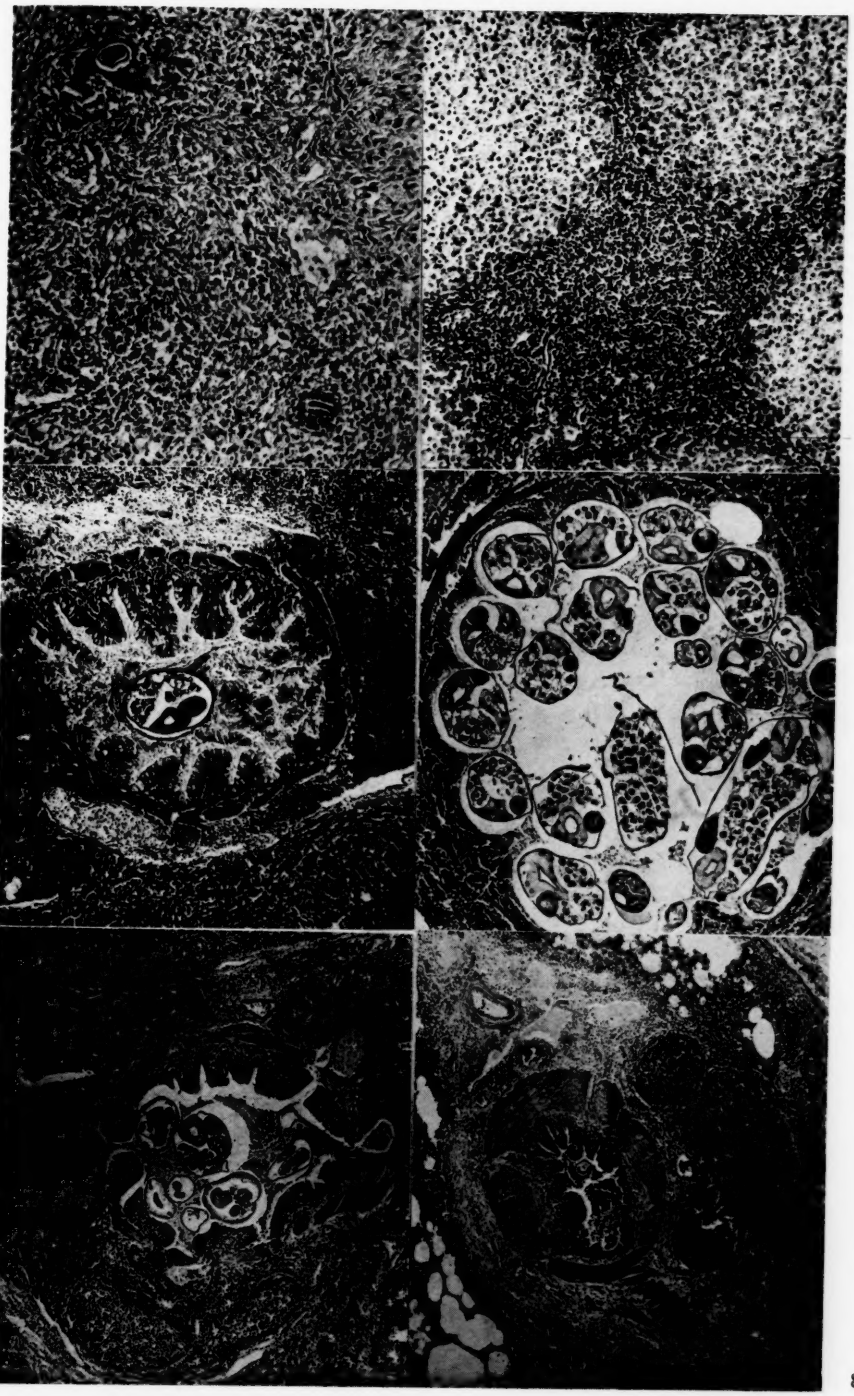
PLATE II



3 4

5 6

7 8

(*Article by Dunn, page 327*)

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Up to the time of dosing, the eosinophil count in this pig never rose above 3 per cent. Two weeks after dosing it rose to 14 per cent, and then fell to a level of 1.5 to 4 per cent in succeeding weeks.

Pig M7. This was the control pig right up to bacon weight. It made good weight gains until the 18th week although it was usually slightly below the average of the litter. During the 19th week the animal went off its food, showed scouring, and had a temperature of 105° F. It was isolated from the rest of the litter and was given penicillin and sulphamezathine for three days. It recovered slowly and was returned to its litter mates after three weeks in isolation. Its subsequent growth was satisfactory, but the setback it suffered caused it to be 25 lb. below bacon weight at 27 weeks of age. The lungs and liver were macrospocically normal and there were no lungworms present. No lungworm eggs were found in the fæces at any time, and eosinophil counts were within the range of 2 to 5 per cent, except on one occasion, when the count was 8 per cent.

Discussion

It was fortunate that in the main experiment the sow had a very low parasite burden and none of the litter of seven pigs ever showed helminth eggs in the fæces, apart from the lungworm eggs which appeared after they were experimentally infected with lungworm larvæ.

The preliminary experiment was complicated by natural lungworm infection in the two litters from which the three experimental pigs came, the existence of which was discovered accidentally when one pig had to be killed because of injury.

Dosing with infected earthworms was very successful in producing infection, for each of the six pigs dosed in the main experiment (M1 to M6) showed lungworm eggs in the fæces and adult lungworms at autopsy.

Dosing with lungworm eggs produced little effect on the health of the pigs, no matter what the dose, or whether given as single or repeated doses. The only exception was pig M3, which received 16 weekly doses of 500 larvæ each and failed to gain weight as fast as its litter mates. Coughing occurred in most of the pigs at about three weeks after dosing, but, with the exception of pig M4, it quickly disappeared. No pig showed lung lesions which were not attributable to lungworms: no lesions resembling those caused by virus pneumonia were seen in any of the pigs on the premises.

Some evidence of the lesions caused by migrating lungworm larvæ was seen in pigs P1 and M1 in the form of necrotic foci in the intestinal lymph nodes and lungs, with heavy eosinophil infiltration. There were "milk spot" lesions in the liver of four of the six pigs infected in the main experiment, and it seems likely that these were caused by migration of lungworm larvæ by a route other than the normal one. These lesions consisted of periportal accumulations of eosinophils, with fibroblast proliferation, and were identical with those generally attributed to migrating *Ascaris* larvæ (Oldham and White, 1944). No larvæ were seen in these lesions, either in fresh preparations or in histological sections: larvæ are also rarely found in *Ascaris* "milk spot" lesions.

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The lung lesions seen in the experimental pigs at bacon weight closely resembled those observed in natural infection of bacon pigs (Dunn, Gentles and White, 1955), a typical example of which is shown in Fig. 2. They comprised greyish, lymphoid nodules and lobular areas of emphysema associated with hypertrophy of the muscle of the bronchioles, which are often blocked by one or more adult parasites (Fig. 8). These lesions were mainly located in the diaphragmatic lobes, especially at their posterior tip and along their inferior border. The lymphoid nodules resemble those often found in the portal tissue in "milk spots" in the liver following *Ascaris* migration (Oldham and White, 1944).

In comparing the effects of dosing with lungworm it is best to exclude the preliminary experiment, since the two litters from which the three pigs, P1 to P3, came contained naturally infected pigs, and so although the control pig (P3) was free from infection it is possible that the infection in P2, like that in P1, might have been the result of natural infection.

The six pigs dosed in the main experiment (M1 to M6) received a wide variety of doses at different ages, and it is difficult to compare the results in the individual pigs. One comparison that can be made, however, is between pigs M1 and M4, on the one hand, and M2 and M5 on the other. The first two received 4,000 to 5,000 larvæ when very young (two to eight weeks); they gave very high egg counts (3,100 and 7,750 e.p.g. at the peaks) and had high worm burdens at autopsy. In contrast, pigs M2 and M5, which were first dosed when 12 to 13 weeks old (3,100 and 20,000 larvæ), both showed low egg peaks (7 and 31 e.p.g.), and both had low worm burdens (13 and 1) at autopsy at bacon weight. Comparison of these two pairs suggests that the older pig is more resistant to infection, either because fewer larvæ develop into adults or because more adults are expelled, or for both reasons. Egg counts and residual infections may thus be less in pigs infected first at three to four months, although it is obviously not possible to state this with any certainty without further evidence.

Pigs M1 and M4 would seem to represent the highest degree of biological success for the parasite, for in both pigs there were large numbers of female worms and high egg counts. Pig M1 was killed early, and so it is not known to what extent the host would have succeeded in expelling the worms, but M4 grew well to bacon weight and yet harboured a very large number of adult lungworms which produced a total of many millions of eggs. In most of the experimentally infected pigs, and, in our experience, in most naturally infected pigs also, the resistance of the host succeeds in reducing the parasite burden to a low level, and thus reduces the degree of infection of the earthworm population on pasture. Only under certain conditions, as when young suckling pigs pick up a very heavy initial infection, and especially when such factors as weather, housing or bacterial and virus infection also act against the host, are severe clinical verminous pneumonia and deaths likely to occur. For the most part the pig has become very successful in keeping down the level of infection and is able to tolerate moderate lungworm burdens, with steady egg production over periods

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of many months, without serious adverse effects on general health or body weight.

Lungworm eggs first appeared in the fæces three to four weeks after dosing, and the peak of egg production occurred five to nine weeks after dosing. There was usually a fairly steep fall in egg production after the peak was reached, and after a few weeks it remained at a level which bore a fairly clear relationship to the total population of female worms found at slaughter. This agrees with our findings in a series of 14 bacon pigs with a natural infection, where the egg production was within the range of 0.03 to 0.75 e.p.g. fæces for each female worm. In four of the six experimentally infected pigs allowed to survive to bacon weight, the corresponding figure for the four weeks before slaughter was 0.075 to 0.37 e.p.g. fæces per female worm, which represents a total daily output in the fæces of some 150 to 740 eggs from each female worm (Table 2). This relatively

TABLE 2 Relation between egg production and population of adult female M. apri in experimentally infected pigs killed at bacon weight

Pig No.	Mean e.p.g. for 4 wks. before slaughter	No. female M. apri present	E.p.g. per female M. apri
P2	1.5	15	0.10
M2	1.1	3	0.37
M3	0.12	22**	0.005
M4	170	998	0.17
M5	12	1*	12
M6	0.3	4	0.075

* Additional worms found in histological sections

** Some were immature

constant relationship is remarkable when one considers that lungworm eggs have to be coughed up and swallowed before appearing in the fæces. Once the peak of egg production is over, egg counts remain very similar from day to day and week to week. It is sometimes suggested that egg counts in individual animals are so erratic as to be unreliable, but the evidence in the case of *Metastrongylus apri* in the present experiment, as well as in naturally infected pigs, suggests that fæcal egg counts may show remarkable constancy and may also be fairly reliable indicators of worm burdens.

Some of the features of egg production are illustrated in Fig. 9, which shows the fæcal egg counts of Pig M6 which received a single dose of 500 lungworm larvæ in the form of infected *E. fætida* when 18 weeks old. For comparison, there are values for a pig (N) which developed a natural infection as a result of grazing a small grass plot at the Field Station for a period of one week just after weaning. This was the only period during which the animal came into contact with earthworms. The degree of infection of the plot was only

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slight, since only six of 30 earthworms examined showed lungworm larvæ and all infections of individual earthworms were light.

Three weeks after dosing, Pig M6 showed lungworm eggs, and the peak of egg production (50.5 e.p.g.) occurred two weeks later. There was then a sudden fall to 10 e.p.g. and low values until slaughter at 28 weeks. At autopsy there were four female lungworms, representing an egg output of 0.075 e.p.g. per female over the three weeks before slaughter.

The naturally infected pig was exposed to infection at a much earlier age and the egg production remained near the peak (28 to 38 e.p.g.) for five weeks and then fell steeply to low resting levels. At slaughter there were 23 female worms, representing a final egg production of 0.2 e.p.g. per female worm. These levels of egg production and worm burden are typical of those found in a number of pigs exposed to similar degrees of natural infection on this and other farms, and probably represent the most common level of natural lungworm infection.

The cause of the steep fall in egg counts that occurred in most pigs might be either reduced egg laying by a constant number of lungworms or expulsion of adult worms, or both. The evidence presented in an earlier communication (Dunn, Gentles and White, 1955) suggests that in natural infection there is usually expulsion of lungworms from most parts of the lungs except the posterior part of the diaphragmatic lobes, whereas in young animals the worms are found fairly evenly distributed throughout the lungs. Pigs M1 and M4 both showed the even distribution of lungworms characteristic of heavy natural infection in young pigs, whereas the other experimentally infected pigs showed lungworms mainly confined to the predilection sites of " residual " infection. Pig M1 was only 9 weeks old when slaughtered and represented the state of affairs we have found in heavy natural infection of the young pig killed just after weaning. Pig M4, however, still carried a heavy infection of adult worms when killed at bacon weight and was for some reason unable to expel its adult worms. It possibly never had many more adults than the 1,488 present at slaughter since it only received 5,000 larvæ and one would not expect all of them to develop into adults.

Good feeding and housing may well have accounted for the lack of more marked symptoms in the infected pigs.

Summary

Eight pigs were fed single or repeated doses of infective larvæ of *Metastrongylus* apri in the form of infected specimens of the earthworm *Eisenia fœtida*. Fæcal egg counts and differential leucocyte counts were carried out during the life of the pigs, the lungs of all the pigs were examined macroscopically and histologically at slaughter, and the numbers of lungworms present were recorded. Two uninfected pigs were used as controls.

All the dosed pigs developed lungworms. The eggs were demonstrated in the fæces by magnesium sulphate flotation and usually first appeared three to four weeks after dosing. Egg production rose rapidly to a peak and then usually fell to a low and relatively constant level of between 0.075 and 0.23 eggs per

gramme of fæces per female worm. The only symptom observed was coughing, and there was no interference with growth and weight gain, except in one pig.

Blood eosinophils usually rose two weeks after dosing to a level of 10 to 15 per cent and then fell to 2 to 6 per cent if no further doses of infective larvæ were given.

The lung lesions seen at autopsy comprised a verminous pneumonia with giant cells, associated with the recent development of the parasites to the egg-laying stage and the liberation of large numbers of eggs into the lung parenchyma, and later the more chronic lesions of bronchitis, emphysema, peribronchial lymphoid hyperplasia and bronchiolar muscular hypertrophy in animals that were not killed until at least nine weeks after dosing. Greyish nodules up to 8 mm. in diameter, formed from peribronchial lymphoid hyperplasia and situated mainly along the inferior border of the diaphragmatic lobe and at its caudal end, were the characteristic gross lesions in adult pigs. These lesions are identical with those found in naturally infected pigs.

Acknowledgments

I thank Professor E. G. White for his criticism of the manuscript and his advice throughout the work, Miss Moya Gentles for careful technical assistance and for some of the photographs, Messrs. Flemming and Irvine for the photographs of the lungs, and Mr. R. B. Griffiths for helpful advice.

The work was carried out with the aid of a grant from the Agricultural Research Council.

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XUM

ARTIFICIAL INSEMINATION IN DOGS

The First Transatlantic Conception

By A. E. HARROP, M.R.C.V.S.

Animal Husbandry Department, Royal Veterinary College, London

Introduction

As previously described by the author (Harrop, 1954*b*), artificial insemination of dogs, using preserved semen, can be accomplished successfully. A bitch at the Royal Veterinary College, London, was inseminated with semen over 100 hours old and whelped two male puppies.

Further work revealed that this preserved semen was capable of withstanding the hazards of travel. Using a specially designed polythene holder in a thermos flask, samples of preserved semen, packed in crushed ice, were sent to Glasgow from London by rail, held there for 24 hours, and then returned. On return they were examined microscopically and showed little deterioration after being stored in this manner for four days and travelling some 700 miles.

A practical test was carried out in April, 1955, when a sample of preserved semen was sent by road and rail to the Canine Research Station, Animal Health Trust, Newmarket, where it was inseminated into a bitch which subsequently produced eight puppies after a 63-day pregnancy (Fig. 1).

Encouraged by these successes a more ambitious programme was designed. It was to attempt to transport canine semen to the U.S.A. with a view to obtaining a conception in that country. A description of the operation is appended.

Technique

Collection and preservation of the semen.

All semen samples were collected in fractions from the dogs used in these experiments with the aid of canine artificial vagina (Harrop. 1954*a*). The second fraction of the ejaculate—the sperm-bearing fraction—was isolated and diluted with heat-treated pasteurised milk as described in previous publications (Harrop, 1954*b*; Harrop, 1955) (Fig. II).

Transport of the semen.

The semen fraction, diluted 1 :8 with heat-treated pasteurised milk, was placed in a sterile glass tube which was wrapped in cotton-wool. This tube was inserted into a larger glass vessel which was in turn clipped on to the polythene holder, and the whole fitted into a thermos flask. The flask was filled with crushed ice, and in this manner the semen sample was kept at about 4° C. for the duration of the journey. The object of the polythene holder was to try and eliminate any undue vibration on the journey (Fig. III).

The journey to New York State Veterinary College, Cornell University, Ithaca, N.Y., U.S.A., where the bitches were kept, was of many thousands of

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PLATE I

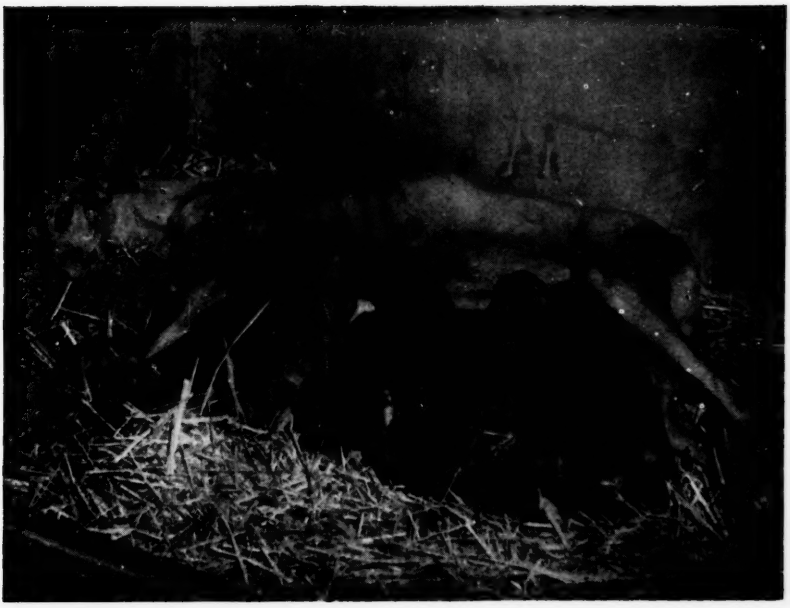

Fig. I
Bitch and puppies born at the Canine Research Station, Newmarket.
Pure-bred greyhounds.

Fig. II
Collecting semen from a beagle dog.

(*Article by Harrop, page* 338)

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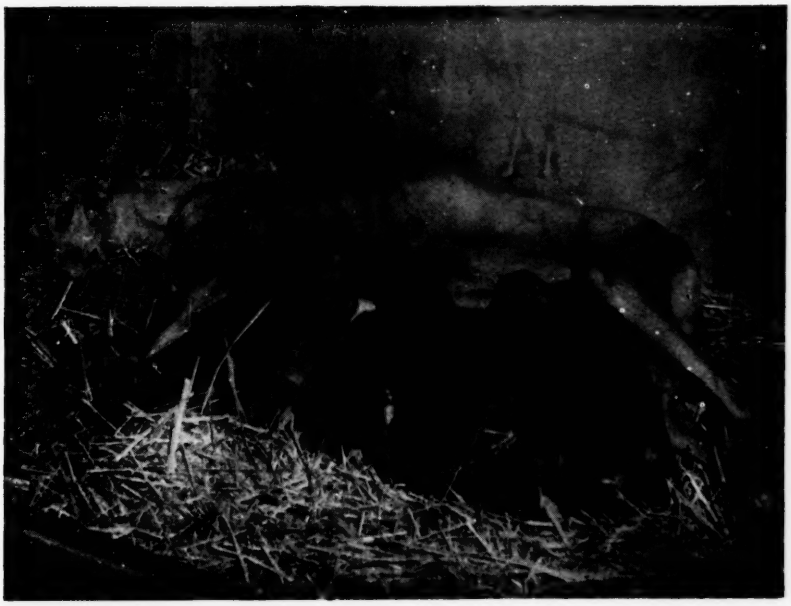

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Collecting semen from a beagle dog.

(*Article by Harrop, page* 338)

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PLATE II

Fig. III
Thermos flask of type used in these experiments, together with polythene holder. Semen sample may be seen in the inner tube.

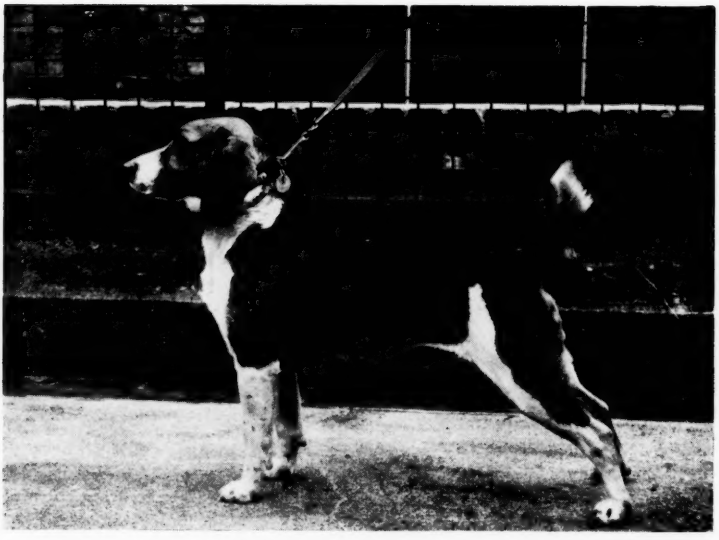

Fig. IV
Mr. D. H. Appleton's "North Hertfordshire Faerie," sire of the litter born in the U.S.A.

(*Article by Harrop, page* 338)

miles and included three changes of aircraft. In spite of the utmost co-operation from the B.O.A.C. and the American airlines, the shortest time in which the semen could reach its destination was about 48 hours.

In this manner seven semen samples were flown to the U.S.A. over a period of some twelve months. The first of these was not used for insemination purposes, but merely to ascertain if the experiment was practicable. Of the other six samples. one was non-motile on arrival due to faulty preparation and the other five were inseminated into bitches.

Insemination.

One of the greatest difficulties associated with these experiments was that of attempting to assess in advance the correct time at which to inseminate the bitch. Vaginal smears were taken from the bitches and examined in order to assist in this procedure, but, because of the two to three days required for the journey, plus the 24 hours necessary to collect and prepare the semen and reserve airway accommodation, it meant forecasting the correct stage of œstrous almost a week in advance. These factors are possibly responsible for only one of the five bitches inseminated proving pregnant.

The semen examinations, the taking of the vaginal smears and the actual inseminations, were carried out by Professor E. P. Leonard and his colleagues at the New York State Veterinary College.

The bitch which conceived was a pure-bred Beagle, and the semen used was also taken from a pure-bred Beagle.

The semen was despatched from England per B.O.A.C. on April 19, 1956, in response to a cable from Professor Leonard.

Professor Leonard reports: "The bitch was inseminated on April 25, the semen then being some 140 hours old, and the motility of the semen at the time of insemination was 30 per cent. The gestation period was 66 days, the bitch whelped normally on June 30, and the litter was of five healthy puppies, three females and two males. The dam and the puppies are progressing well" (Fig. IV).

Discussion

While artificial insemination has long been recognised as a method of breeding amongst the domesticated animals, and although the dog was the first subject used in such experiments, breeding by artificial insemination in dogs has not been persisted with other than for experimental purposes. The reason for this has been the difficulty in finding a suitable medium in which to preserve the semen for the necessary length of time.

This obstacle has now been removed, and these experiments show that it is possible not only to inseminate bitches in this country but also to export the semen and achieve conceptions abroad. In this way an international exchange of canine strains could be facilitated in a manner heretofore impossible because of distance factors and/or quarantine restrictions imposed by certain countries.

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It must not be inferred from this report that long-distance artificial insemination in dogs is now a practical proposition and can be embarked upon with impunity. It must be remembered that only one out of five bitches inseminated conceived, although some of the other four bitches were inseminated under similar favourable circumstances and with what appeared to be a better-quality semen sample.

It is obvious that more research and investigation on semen preservation, knowledge of the œstral cycle of the bitch and insemination techniques are necessary to achieve further progress.

Summary

A brief description is given of various conceptions obtained in bitches by artificial insemination using preserved semen, culminating in a transatlantic pregnancy.

Acknowledgments

I wish to thank Professor N. J. Scorgie for his permission to carry out this work, and for his helpful advice and criticism.

I am also greatly indebted to: (*a*) The Director and staff of the Canine Research Station, Animal Health Trust, Newmarket, for their co-operation in insemination trials. (*b*) Professor E. P. Leonard and his colleagues of New York State Veterinary College, Cornell University, Ithaca, N.Y., U.S.A., for examining the semen samples and inseminating the bitches; (*c*) The Gaines Dog Research Center. 250 Park Avenue, New York. U.S.A., without whose financial help and professional interest this venture might not have been achieved. (*d*) Mr. D. H. Appleton, Appeline Kennels, Baldock, Herts., for the loan of his dog, "North Hertfordshires Faerie," which was the sire of the successful insemination.

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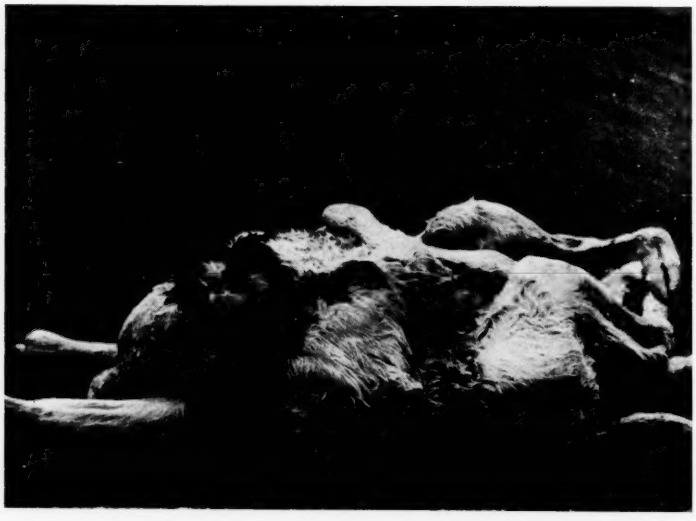
A case of *Cephalothoracopagus monosymmetros* in the bovine species. C. Viewed from below.



D. Viewed from the left.

(*Article by Dransfield and Pinsent, page* 341)

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THE ANATOMY OF A CASE OF *CEPHALOTHORACOPAGUS MONOSYMMETROS* IN THE BOVINE SPECIES—PART II

By J. W. DRANSFIELD

Department of Veterinary Anatomy, University of Liverpool

In the type of case represented by the specimen, incomplete twinning has occurred which has resulted in a single anterior extremity and double posterior extremities, the duplicated extremities being in part united along their ventral aspects, and in part separate. (An ingenious schematic representation of the various planes in which twinning can take place is given by Wilder [1904]). The most complete degree of separation in such cases of what might be called ventral union is present in the well-known "Siamese Twins," where two individuals, completely separate in all other respects, are united by a small area of abdominal wall, often occupied by a bridge of liver tissue. In *Cephalothoracopagus* the separation is much less complete. The hindquarters are completely distinct but anterior to the umbilicus the abdominal, thoracic and cervical regions are united along their ventral aspects, producing common abdominal and thoracic cavities. In the region of the head the two individual elements are united in a similar manner but with variable effect, depending on the degree of obliquity at which the two heads meet.

In *Cephalothoracopagus disymmetros* a common head is formed with two faces, which are, however, composite. The right half of the head of one individual element and the left half of the head of the other unite to form a complete face (which may be said to be dorsal or ventral) and vice versa. In Fig. 1 this is shown diagrammatically, S (Sinister) representing the left individual element and D (Dexter) the right. An alternative title for this type where two distinct composite faces are present is, of course, Janiceps, i.e., with head similar to Janus.

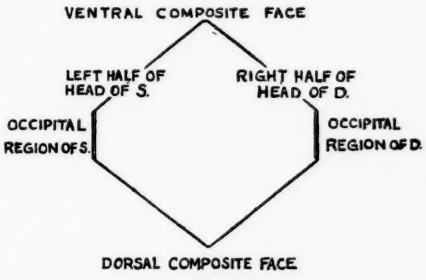
VENTRAL COMPOSITE FACE
LEFT HALF OF HEAD OF S.
RIGHT HALF OF HEAD OF D.
OCCIPITAL REGION OF S.
OCCIPITAL REGION OF D.
DORSAL COMPOSITE FACE

Fig. 1
Diagram of formation of head regions in cases of *Cephalothoracopagus disymmetros.*

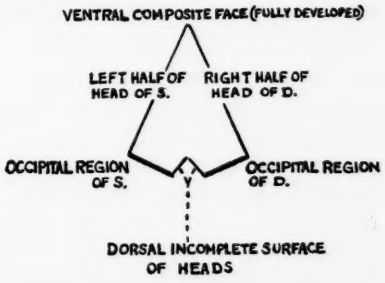
VENTRAL COMPOSITE FACE (FULLY DEVELOPED)
LEFT HALF OF HEAD OF S.
RIGHT HALF OF HEAD OF D.
OCCIPITAL REGION OF S.
OCCIPITAL REGION OF D.
DORSAL INCOMPLETE SURFACE OF HEADS

Fig. 2
Diagram of formation of head regions in cases of *Cephalothoracopagus monosymmetros.*

In *Cephalothoracopagus monosymmetros,* the more common occurrence, the heads are united at varying angles, one composite face being complete, the other showing degrees of suppression varying with the angle of union. In the

great majority of cases, all that remains of the faces and the corresponding parts of the head on the incomplete side, superficially, are the two ears, and these may be absent. This arrangement, which was present in the subject to be described, is shown diagrammatically in Fig. 2. Superficially the head appears normal except for its excessive breadth and for the presence of the two, usually conjoined, ears on the incomplete side. The face, which is apparently normal, is in fact composite, formed by the left half of individual S and the right half of individual D.

As the various structures of the body are described it will be seen that there is a tendency for these to be composite in all cases where they are situated on the ventral aspect of the individual elements.

It may be mentioned here that the terminology of such monsters always presents a difficulty. Many of the workers who have studied the various monsters have evolved their own terms with little attempt to achieve a standard terminology. Such an attempt was made by Schwalbe (1907), and as his is the only really complete study of monsters, apart from a recent work by Wolff (1948), the terminology used here is that of Schwalbe. A summary of the terminologies of the earlier workers, Gurlt and Isidore Geoffroy Saint-Hilaire is given by Craig (1912).

External Appearance of the Monster

In the present case the subject was a female calf, weighing 67 pounds, progeny of a Friesian heifer. As pointed out in Part I of this paper the fœtus had been removed in a number of different portions after embryotomy. However, these had been reassembled so efficiently at the Field Station that no difficulty was encountered in visualising the fœtus as it had been before the operation commenced. The general arrangement will be seen from the photographs accompanying the paper. The single head was clearly broader than usual, especially posteriorly, and, in addition to the two external ears in the normal position, two flattened external ears, apparently continuous with one another, lay in the middle line of the occipital region. A small opening between these ears proved to be a common ear canal. Leading posteriorly from the head were two distinct cervical regions. The two thoraces, each bounded by thoracic vertebræ and ribs, were continuous at what were the ventral aspects of the individual elements, forming one large common thoracic cavity (the actual disposition will be dealt with later). Lying in the usual relationship to each thoracic wall was a forelimb, there being four in all. The right limb of individual element S and the left limb of D crossed one another on the upper surface of the monster, while the left limb of S and the right limb of D were directed forwards, these latter being obviously the limbs originally presented in the maternal vagina. In order to attain this position considerable distortion had occurred in the shoulder regions, each being bent laterally through about 90 degrees, but it is thought that this would be a secondary effect due to the limbs accommodating themselves on the sides of an abnormally broad thoracic region.

Anteriorly to the umbilicus a common abdominal cavity was present, formed by the union of two individual cavities along their ventral walls, each having its

own elements, including the lumbar vertebræ. Immediately posteriorly to the umbilicus, the two individual elements became completely separated from one another. All structures were apparently normal. Each element had normal mammary glands with four teats, normal external genitalia, a normal tail and two hindlimbs. The only abnormalities associated with the hindlimbs were slight medial distortions of the metatarsal and digital regions, most marked in the left limb of individual D, less marked in the right of D and the left of S, and absent in the right of S. Such limb distortions appear to be relatively common in monstrosities where there is involvement of the nervous system in any way. In the human subject, limb deformities are said to be present in some cases of hydrocephalus (Ballantyne, 1904; Williams, 1931), and it is interesting to compare the cases of *Cephalothoracopagus* in the human described by Rohrbach (1952) in one of which club feet were present. It has been suggested that absence of normal intrauterine fœtal movement, due to the derangement of the nervous system, may result in these distortions of the limbs.

Reference must be made to the *umbilicus*. During the manipulations for the delivery of the monster the umbilical cord had broken off short and there was no indication of whether it was single and no indication of the number of vessels contained in it. The umbilicus itself was single and within the monster each individual element showed two umbilical arteries and one umbilical vein connected with the umbilicus. Schwalbe (1907) states that the umbilicus is single in all cases of partial duplication with ventral union, except for *Xiphopagus,* in which the bodies are only joined in the xiphoid regions.

Skin, Hair and other External Features

The skin, with a plentiful covering of hair, appeared normal in every respect. The hair showed the usual black and white colouring of the breed, and even a casual glance was enough to show that wide differences existed between the colour patterns of the two individual elements. It would appear to be of great significance that incomplete twinning, which results from the incomplete fission of one ovum, should produce conjoined individuals having such widely different colour patterns.* One may mention here that the buttocks of the two elements showed black patches which were quite different from one another. A very detailed examination of the patterns for indications of correspondence of shape and position of black patches, however slight, was made and this suggested that the individual elements were reciprocal twins and not mirror images of one another, this view being further substantiated by the arrangement of the internal organs. It must be emphasised that any such similarity was very slight and in some areas was non-existent. Landtwing (1939), dealing with a monster showing anterior duplication, made a very close study of the patterns of hair streams and vortices, but as the important ones are in the head region, they are of no assistance in the present case. The body hair patterns were poorly marked and had been involved in some of the embryotomy incisions. In connection with the dissimilarity of conjoined twins, Schwalbe (1907) quotes Strassmann in pointing

* In this connection the paper by J. Hancock (1952), "Identical cattle twins and colour inheritance" (*Endeavour, 11,* 78-86), is of interest.

out that similarity of uniovular twins is no greater and often not so great as that of biovular twins, while Schwalbe himself states that *Situs inversus* is very rarely described in one of the members of a pair of twins.

It will be convenient to mention here that the two eyes and their associated structures were normal, as were the nostrils. The external ears on the lateral surfaces of the head had the usual appearance, but on the upper aspect of the head were the two conjoined external ears of the incomplete surface of the head. The two conchal cartilages of these appeared rather flattened, lying on the upper aspect of the head. They guarded the opening into the single common ear canal and were continuous with one another in the middle line of the monster. Deeply, each had a downward projection embracing the single annular cartilage, which, however, had the appearance of being derived from the coalescence of two separate cartilages. There were two scutiform cartilages which were typical in shape, but were rather small. The ear muscles, allowing for the distortion present in this region of the skull, were in the usual arrangement.

Skeletal and Muscular Systems

It is thought that the descriptions of the remaining systems will be more readily understood if the skeletal peculiarities are now described, with an indication of the principal features of the muscular system.

1. Skull.

The skull, which was apparently single in its anterior part, became completely double posteriorly with a foramen magnum on each side, each bounded by two occipital condyles. This arrangement, which provided the necessary articular areas for the two vertebral columns as they met the skull, was produced by the oblique union of the heads already mentioned and shown in Fig. 2. The facial region of the skull, apparently single, was, in fact, formed by the left half of the facial region of individual element S and the right half of the facial region of D. Between the occipital regions of S and D was an area formed by the conjoined incomplete surfaces of the skull elements of S and D. This area

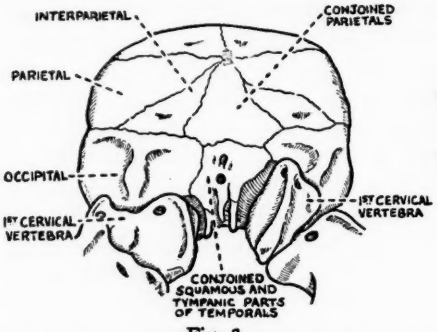

Fig. 3
Drawing of posterior aspect of cranium with associated 1st and 2nd cervical vertebræ.

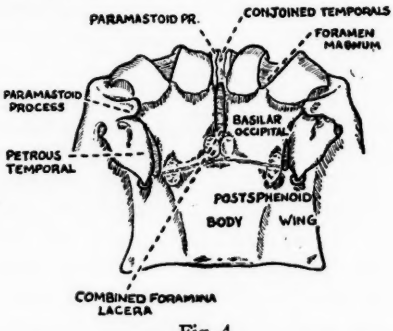

Fig. 4
Drawing of posterior part of base of cranium.

is shown in Fig. 3. It will be seen that, although the frontal bones were single, the parietal and interparietal elements were doubled, the axial parietal bones

having coalesced. Below these conjoined parietal bones lay the united squamous temporal bones. These were considerably compressed from side to side and had fused with the tympanic parts of the petrous temporals. This produced an irregular surface of bone showing two openings in the middle line. The upper was the common orifice of the parieto-temporal canals, and the lower the single opening of the canal of the external ear. There were clearly two tympanic parts of the petrous temporals, but these were very much compressed around this single ear canal, which led down to the single narrow tympanic cavity. Within this cavity there was a single series of auditory ossicles, the stapes being attached to a duplicated fenestra vestibuli. The internal ear was completely double, each being contained in a petromastoid part of the temporal which showed much less compression. Clearly the greatest compression and distortion had affected the tympanic parts. The single stapes was of interest as the corresponding hyoid elements (associated with the stapes in development) were double. However, the ossicles were much distorted, and it was difficult to be certain that the stapes did not exhibit a degree of duplication. There were traces of one tensor tympani and two stapedius muscles in the cavity.

The case of Vrolik cited by Schwalbe (1907) shows rather a similar degree of oblique union in the skull in the human subject and, as would be expected, there are similarities in the anatomy of the brain in Vrolik's case and the monster under discussion.

One of the most surprising and, it seems, unique features of the present case concerned the petro-mastoid parts of the temporal bones in this region. Taking origin from the cleft between the cranial aspects of the two bones and from part of the tympanic segments was a small cylindrical mass of muscle lying within the cranial cavity, but external to the dura mater. This structure narrowed anteriorly and split into one main central tendon and several subsidiary ones, all of which ended in an indefinite manner in the dura mater immediately behind the hypophysis (Fig. 7). A number of plexiform blood-vessels surrounded this muscle, which received its nerve supply from both axial seventh cranial nerves and both axial fifth cranial nerves, i.e., the seventh and fifth cranial nerves of the two incomplete surfaces of the conjoined heads. It was considered probable that this muscle mass, taking into account its nerve supply, origin and general disposition, represented a number of the muscles of the jaws and of the hyoid apparatus which had coalesced. The remarkable feature is the persistence of this muscle mass within the cranial cavity.

Compression of the various structures in the middle line of the skull, associated with varying degrees of coalescence has been mentioned. It is a well-known fact that in these double monsters the fission may have produced the precursors of two perfectly normal structures, one on each side of the middle line, but later, unequal growth, especially in the axial direction, produces distortion, compression and also coalescence in the actual structures.

Fig. 4 shows the appearance of the base of the skull in the region of duplication, and the compression and distortion of the petrous temporal bones may be seen axially. The basilar part of the occipital bone was double, the

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postsphenoid being single. Between the two basilar parts was a cleft, the combined foramina lacera of the incomplete surfaces, appearing as a single opening superficially which became two distinct openings deeply.

The hyoid apparatus related to the main part of the head was normal, but posteriorly, between the two sets of occipital condyles, a very much compressed hyoid apparatus supported a rudimentary tongue and a normal larynx.

The remaining point of interest in connection with the skull concerns the palate of the main part of the head. This was completely cleft, the palatine processes of the maxillæ and the corresponding parts of the palatine bones being represented by slight ridges, the buccal and nasal cavities being continuous and the tongue lying between the borders of this wide cleft.

2. Cervical Regions.

Apart from a certain degree of distortion, most of the vertebræ were normal. The distortion was presumably caused by the unnatural curve undertaken by the bones in running from the region of the skull to the two series of thoracic vertebræ bounding the common thoracic cavity. It both necks the distortion had produced a coalescence of the second and third vertebræ on one side of the neck while they were distinctly separated by the intervertebral disc on the other. A similar, but more marked, malformation of vertebræ in a double monster is noted by Hughes (1946).

3. Thoracic Regions.

Just as in the regions of the conjoined heads, the thoracic regions showed a considerable continuity. The common thoracic cavity was bounded by two series of thoracic vertebræ, two paired series of ribs and two composite sterna (Fig. 5), but an important distinction from the head region lay in the fact that

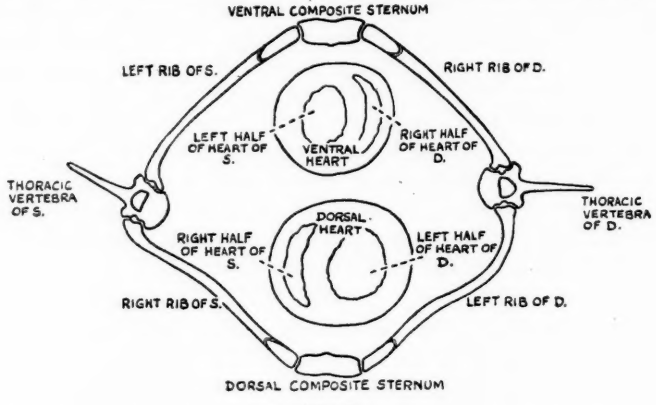
VENTRAL COMPOSITE STERNUM
LEFT RIB OF S.
RIGHT RIB OF D.
LEFT HALF OF HEART OF S.
VENTRAL HEART
RIGHT HALF OF HEART OF D.
THORACIC VERTEBRA OF S.
THORACIC VERTEBRA OF D.
DORSAL HEART
RIGHT HALF OF HEART OF S.
LEFT HALF OF HEART OF D.
RIGHT RIB OF S.
LEFT RIB OF D.
DORSAL COMPOSITE STERNUM

Fig. 5
Diagram of formation of common thoracic cavity and of two hearts.

the composite surfaces were complete. Assuming that the axes of the two individual elements were right and left in position, it will be seen that the composite sterna were dorsal and ventral. The thoracic walls were rather distorted,

more especially those placed dorsally, but otherwise were quite normal, although the breadth was unusually wide. The anterior extremities of the sterna gave origin to the usual cervical muscles, those from the dorsal sternum passing to the incomplete composite side of the head, while those from the ventral sternum ran to the fully-developed side of the head.

The diaphragm was a single structure closing the thoracic cavity posteriorly, but there were clear indications that there were present the components of two diaphragmata united to one another. Centrally, it was rather a thin, delicate structure and in the part which was lying in individual element S there was a large opening, 15 cm. by 7 cm., through which part of the rumen and part of the spleen of S projected into the pleural cavity on the left of the ventral sternum.

According to Schwalbe (1907) such diaphragmatic defects occur commonly in cases of *Cephalothoracopagus*, but he states that they are present most frequently on the incomplete surface. In the present case the opening was in relation to the ventral sternum, which corresponds with the complete surface of the head. Gruber (1927) states that diaphragmatic defects are frequently present in double monsters and that left-sided defects are much more common than those on the right side in all types of malformation of the diaphragm.

4. Remainder of the Skeletal System.

The remaining parts of the two axial skeletons and the associated muscles were completely normal, as were the skeletons of the limbs and the associated muscles, apart from the deviations of the limbs which have already been described.

This article will be concluded in the September (Special Congress) issue. References will be included.

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REVIEW

SYMPOSIUM ON MEDICATED FEEDS, by Welch and Marti-Ibanez. Medical Encyclopedia, Inc. Price, $4.00.

THIS symposium, which was sponsored by the U.S. Department of Health, Education and Welfare, Veterinary Medical Branch, and presented in January. 1956, is a collection of over 30 papers covering the many factors that arise from the addition of drugs to food. The drugs discussed include antibiotics, hormones, anthelmintics and arsenicals. The different papers deal with the history of medicated foods, economic aspects, measurement of quantities in the food and in the tissues of the animal after feeding, legal responsibility, dangers to health and the future of medicated foods in the livestock industry.

The articles are reproduced in full with tables and bibliography, and the book ends with a panel discussion on questions arising from the symposium.

This symposium can be recommended as a progress report on medicated foods seen through American eyes at the present time when their future is still "in the melting pot." So many drugs which have appeared at first sight to be wholly favourable have, on further research, proved to possess undesirable side effects when put to practical test. The authors in this symposium present the facts and some opinions—future research alone can give a balanced judgment.

NEWS

BAYER PRODUCTS LTD. and its associated exporting company, Winthrop Products Ltd. announce the construction of a new factory near Newcastle-on-Tyne, at which manufacturing facilities will be concentrated. The factory will be operated by a new manufacturing division: Winthrop Laboratories Ltd., and it is expected to come into operation towards the end of 1956. The administrative and other offices of Bayer Products Ltd. and Winthrop Products Ltd. will remain at Neville House, Kingston-on-Thames, Surrey, and the Biological Institute will remain at Exning, Newmarket.

IMPERIAL CHEMICAL (PHARMACEUTICALS) LTD. announce that they have introduced "Stromez" Streptomycin/"Sulphamezathine" Dispersiple Powder. "Stromez" contains in each 100 gramme envelope "Sulphamezathine" (Sulphadimidine B.P.) 10 grammes. Streptomycin Sulphate B.P. 2 grammes (1.4 mega units) and Light Kaolin B.P. approximately 86 grammes. The product readily forms a smooth suspension when the required quantity of water is added, and the dosage of the suspension used is calculated at the rate of 100 grammes "Stromez" per 200 lb. animal. A full therapeutic dose of both streptomycin and "Sulphamezathine" can therefore be conveniently administered at the same time. "Stromez" is indicated for the prevention and treatment of enteritis of all species.

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